THE BACTERIAL MICROBIOMES OF SASKATCHEWAN CROPS AND POTENTIAL FOR PLANT GROWTH PROMOTION

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By

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ABSTRACT

Bacteria associated with the rhizosphere and plant tissues may contribute to host crops by providing nutrients, stimulating growth and/or controlling phytopathogens. These beneficial plant × bacteria associations may have a key role in the establishment and production of agricultural ecosystems. This research thesis investigated the bacterial communities associated with canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown in agricultural fields in Saskatchewan and assessed the potential of some of these bacteria for plant growth promotion. Analysis of bacterial communities associated with these crops suggested a selection of the root endophytic microbiome from the rhizosphere. In contrast, endophytic bacteria associated with aboveground plant organs varied greatly among crops, soils and plant organs. Furthermore, Denaturing Gradient Gel Electrophoresis (DGGE) and high throughput-sequencing analyses of baterial profiles in wheat and canola suggested that each crop may select specific bacterial taxa at different plant growth stages and within different plant compartments. *Pseudomonas* and *Stenotrophomonas* were predominant genera in the rhizosphere and root interior of all crops, suggesting a generalist distribution of these bacteria. Relative abundance of specific bacterial groups in the rhizosphere, as well as bacterial Phospholipid Fatty Acids (PLFA) in the bulk soil, were significantly correlated with soil pH, silt and organic matter content. There was, however, no correlation between soil properties and the most abundant endophytic bacterial genera, thus suggesting that soil characteristics may not influence bacterial communities within plant roots. Culturable bacteria (n=157) isolated from the root interior of the studied crops were tested for their effect on seed germination, root elongation and plant growth promotion. A total of nine bacterial strains, isolated from the root interior of field grown crops, stimulated seed germination and root elongation when inoculated to canola and wheat plants, and five of these bacterial strains also promoted shoot growth in canola. Overall, these results suggest the bacterial microbiomes of the rhizosphere and plant tissues were modulated by soil properties and host crop, respectively. In addition, several endophytic bacteria demonstrated potential for use as bioinoculants in agriculture.

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LIST OF ABBREVIATIONS

AAFC: Agriculture and Agri-Food Canada

ACC: 1-aminocyclopropane-1-carboxylate

AG: Aboveground plant organs

AMF: Arbuscular mycorrhizal fungi

ANOVA: Analysis of variance

BLAST: Basic local alignment search tool

BSA: Bovine serum albumin

CFU: Colony-forming unit

DGGE: Denaturing gradient gel electrophoresis

FAO: Food and Agriculture Organization of the United Nations

GC: Gas chromatography

Gr-: Gram negative bacteria

Gr+: Gram positive bacteria

IAA: Indole Acetic Acid

NMDS: Non Metric Multidimensional scaling

MRPP: Multi-response permutation procedures

NCBI: National Center for Biotechnology Information

OD: Optical density

OM: Organic matter

OTU: Operational taxonomic unit

PBS: Phosphate-buffered saline

PCoA: Principal Coordinate Analysis

PCR: Polymerase chain reaction

PGPB: Plant growth- promoting bacteria

PLFA: Phospholipid fatty acid analysis

QIIME: Quantitative Insights Into Microbial Ecology

RT: Root

RZ: Rhizosphere

sp.: Species

spp.: Species

TSA: Tryptic soy agar

TSB: Tryptic soy broth

1. GENERAL INTRODUCTION

Agricultural production not only provides food supply for a human population that is expected to reach 9.6 billion by 2050, but also is important for supporting several economic activities in all societies (Parnell et al., 2016). Canada is a global leader in food exports, thus Canadian agriculture production has a significant influence on the global food market. Within Canada, crop production in Saskatchewan is an important economic activity, mainly contributing to the export of grains, oilseeds and pulses (Statistics Canada, 2017). Optimizing the use of fertilizers, water and pesticides is necessary for enhancing crop yield and improving long-term soil productivity. In this context, bacteria associated with crops play important roles for enhancing nutrient uptake, controlling diseases and ameliorating stress (Paul, 2014). Improving the current knowledge on crop × bacteria interactions is critical to enhance the efficiency of agricultural systems, while contributing to sustainable practices to protect the environment.

In crops, bacterial communities may colonize all plant compartments including the rhizosphere, phyllosphere, spermosphere and endosphere. Rhizosphere soil is influenced by the secretion of root exudates, which attract diverse soil bacteria (Hardoim et al., 2008). Bacterial communities inhabiting the rhizosphere may have critical implications for crop growth, soil fertility and environment protection (Bakker et al., 2013). In addition, endophytic bacteria can colonize internal plant tissues for at least part of their life cycle without causing symptoms of disease (Hallmann et al., 1997). Bacterial endophytes may produce beneficial effects on host crops such as increased nutrient acquisition, disease protection and improved tolerance to stress conditions (Hardoim et al., 2015; O'Callaghan, 2016). In contrast, reports on the diversity and function of bacteria colonizing the spermosphere and phyllosphere are still scarce (Lindow and Brandl, 2003; Nelson, 2004; Hardoim et al., 2012; Vorholt, 2012). However, seed-associated bacteria also may influence crop growth, as they may contribute to the primary composition of bacterial communities in the crops (Nelson, 2004). Furthermore, bacteria associated with the phyllosphere have been reported to exhibit beneficial effects on nutrient cycling in the leaves and phytopathogen control (Freiberg, 1998; Stadler et al., 1998; Innerebner et al., 2011).

Studies assessing the diversity of plant associated bacteria are conducted using culture independent and culture dependent techniques. Culture dependent methods allow the isolation of bacterial strains with potential to be used as beneficial inoculants for crops, and to assess possible mechanisms involved in plant × bacteria interactions (Finkel et al., 2017). However, culture dependent methods have a limited capacity to isolate diverse groups of bacterial communities that are commonly associated with crops, because only a small fraction of soil microorganisms is culturable (Kent and Triplett, 2002). Using culture independent techniques also allows identifying common bacterial taxa that are detected in most crops and should be prioritized for further research, inclusion in culture collections, and manipulative experiments (Busby et al., 2017). Recently, the use of high-throughput DNA sequencing techniques has provided a better understanding of the phylogenetic diversity of plant microbiomes and the functional role of bacteria inhabiting various crops (Bulgarelli et al., 2013).

The diversity and abundance of bacteria associated with crops is affected by biotic and abiotic factors (Sturz et al., 1997). Among these factors, crop and soil characteristics may modulate the composition of crop associated bacteria. Crops may select specific groups of bacteria mainly by affecting the composition of roots exudates, which in turn, may influence the bacterial communities in the rhizosphere, and subsequently the colonization of the plant interior (Garbeva et al., 2004; Jones et al., 2009; Berg et al., 2014). However, additional plant factors affecting the bacterial diversity in crops include plant age, plant development stages, root architecture and/or the presence of wounds (Gaiero et al., 2013). In addition, soil chacteristics may also modulate the bacterial communities associated with crops by provinding unique habitats for certain groups of microorganisms, or affecting the physiology of plant roots, which indirecty influence the plant microbiome (Garbeva et al., 2004). Several soil properties such as soil pH, organic matter and texture are reported to influence bacterial diversity in agricultural crops (Sessitsch et al., 2001; Fierer and Jackson, 2006. Kuzyakov and Blagodatskaya, 2015). However, there is no consensus about which of those plant and/or soil factors have the main effect on the establishment of specific microbial populations on the crops (Garbeva et al., 2004).

Beneficial effects on crop growth and/or health suggest that bacteria associated with crops have great potential to become effective microbial inoculants for crops (Bloemberg and Lugtenberg, 2001; Gaiero et al., 2013). Isolation and screening of potential plant growth promoting microorganisms can be a useful strategy to improve for crop management and sustainable

agriculture. Many of these bacteria have the potential to improve crop growth and health by using versatile physiological mechanisms such as nitrogen fixation, phosphate solubilization, production of phytohormones, enzymes and antibiotics (Van Elsas et al., 2006). Recent studies also indicate that the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is a key mechanism involved in the promotion of plant growth (Glick, 2014). In fact, ACC deaminase producing bacteria can increase plant growth when plants are exposed to stress conditions (Reed and Glick, 2005; Glick, 2012). However, typical plant growth promoting mechanisms that are frequently tested *in vitro* often fail to produce an effect on crop yield, thus suggesting that novel mechanisms are still to be determined (Finkel et al., 2017).

Research focused on the diversity and function of bacterial communities associated with crops is important to find practical applications of microorganisms for agriculture. In the current thesis, the following hypotheses were tested:

- Plants growing in different agricultural soils are colonized by unique populations of rhizosphere bacteria and root bacterial endophytes.
- Bacterial communities associated with wheat and canola grown in agricultural soils will differ between plant organs and growth stages.
- Bacterial endophytes isolated from crops grown in different agricultural soils may have the potential to promote plant growth.

The general objective of the thesis was to assess the diversity of bacterial communities associated with Saskatchewan crops and to evaluate their potential for plant growth promotion. The specific research objectives were to:

- Determine the diversity of bacteria associated with the rhizosphere and root interior of wheat, pea, lentil and canola plants grown in different agricultural soils in Saskatchewan using culture-dependent and independent methods.
- Assess the diversity and relative abundance of bacteria colonizing the rhizosphere, roots, shoots and seeds of wheat and canola plants at stem elongation, flowering and ripening stages.
- Assess the potential of bacterial endophytes isolated from agricultural crops to promote plant growth.

1.1. Organization of the Thesis

The following research thesis is organized in a manuscript style. The thesis consists of an Introduction (Chapter 1), Literature review (Chapter 2) and three studies presented in Chapters 3 to 5. The main goal of Chapter 3 was to investigate the diversity of root and rhizosphere bacteria associated with canola, wheat, pea and lentil grown in Saskatchewan agricultural soils using culture dependent and independent techniques including Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rRNA high-throughput sequencing. In this study, a total of 298 bacterial strains were isolated from the root interior of the crops, which were then assessed for plant growth promotion capacity in Chapter 5. In Chapter 4, the bacterial communities associated with the rhizosphere, roots, shoots and seeds of wheat and canola at stem elongation, flowering and ripening were assessed using culture independent techniques. In Chapter 5, the potential of some root bacterial endophytes to enhance crop growth was assessed. Bacterial isolates were tested for their effects on seed germination, root elongation, shoot biomass and plant nutrient content. Additionally, isolates were tested for production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase was examined. Finally, Chapter 6 provides a synthesis of the major findings of the thesis and suggestions for future research.

2. LITERATURE REVIEW

2.1. Plant × Bacteria Interactions: Implications for Crop Production and Agroecosystems Functioning

Agricultural crops interact with a multitude of organisms that potentially may influence plant growth and health. Soil is considered the main source of microbes that form many types of associations with crops. These associations often result in diverse communities of micro- and macroorganisms including bacteria, fungi, protozoa, nematodes, earthworms, ants and moles (Bardgett and van Der Putten, 2014; Frank et al., 2017). Among soil organisms, bacteria have been estimated as one of the most diverse group accounting for up to 10^{10} cells per gram of soil, which represent an estimated diversity of around 5×10^4 species present in a single gram of soil (Roesch et al., 2007; Raynaud and Nunan, 2014). The association between bacteria and plants is mostly mediated by the ability of a plant to release a wide range of organic compounds into the rhizosphere (Smalla et al., 2001; Garbeva et al., 2004). Bacteria colonizing internal plant tissues of both below- and aboveground plant organs also exhibit important functions that impact crop growth (Van Elsas et al., 2006). Complex interactions between the host crop and bacteria occur in the plant–soil interface and the internal plant tissues (Hardoim et al., 2008). Plant × bacteria interactions in the crop rhizosphere/endosphere are deemed beneficial, harmful, or neutral; however, the effect of a bacterial species may vary depending on soil and/or plant growth conditions (Glick, 2014).

Beneficial bacteria that associate with various crops may have important effects in the establishment and development of agricultural ecosystems (Van Elsas et al., 2006). When actively present in crops, some bacteria may increase the availability of plant essential nutrients such as nitrogen, phosphorus and iron. A classical association that occurs between crops and nitrogen fixing bacteria include the symbiotic rhizobia and legume plants. This successful association has been extensively exploited in agriculture for legume production worldwide (Bardgett, 2005). Similarly, additional asymbiotic diazotrophic bacteria including *Gluconacetobacter*, *Herbaspirillum*, *Azoarcus*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*,

Klebsiella and *Pseudomonas* spp. also are reported to fix nitrogen in association with grasses including sugarcane, rice and forage grasses (James, 2000). Nitrogen-fixing bacteria are estimated to contribute a similar amount of nitrogen to soils to that provided by nitrogen fertilizers, accounting for 118 Tg N $year^{-1}$. Leguminous crops provide just over half this amount, an estimated 60 Tg N $year^{-1}$ (Fowler et al., 2013). Similarly, phosphate solubilizing bacteria such as *Pseudomonas*, *Bacillus* and *Rhizobium* spp. also are reported to increases phosphorus uptake by the crops (Rodriguez and Fraga, 1999). In addition to solubilizing phosphorus, *Pseudomonas*, *Bacillus*, *Streptomyces* and *Burkholderia* also are reported to produce low molecular weight iron-chelating compounds called siderophores (Hider and Kong, 2010). These compounds have high affinity for iron in the soil, thus have the potential to increase iron availability for the crops (Chu et al., 2010; Tyc et al., 2017).

Several bacterial species including pseudomonads (*e.g.*, *Pseudomonas fluorescens*, *P. putida*, *P. gladioli*), bacilli (*e.g.*, *Bacillus subtilis*, *B. cereus*, *B. circulans*), and species of *Azospirillum*, *Serratia*, *Flavobacterium*, *Alcaligenes*, *Klebsiella* and *Enterobacter* are reported to promote plant growth in crops (Arshad and Frankenberger, 1997). Many of these bacteria are able to produce plant growth regulators including auxins, cytokinins, gibberellins, ethylene and/or abscisic acid, which have a potential to stimulate plant growth. Plant hormones produced by bacteria are known to influence cell division and root differentiation, thus leading to changes in the root architecture. Depending on the resulting effect on the root system, plant hormones may contribute to enhanced shoot growth (Verbon and Liberman, 2016). An important microbial mechanism involved in the plant growth promotion capacity includes the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick, 2014). The ACC enzyme inhibits the formation of potential high levels of ethylene that can be produced when a plant is subjected to stress conditions.

Crop associated bacteria also play important functions as biological control agents that exhibit antagonistic effects against plant pathogens that cause serious losses in agriculture. In addition, many animal and/or human pathogenic bacteria also may be associated with crops, thus may represent threats for food security and human health (Koberl et al., 2013; Berg et al., 2014; Van Overbeek et al., 2014). Species of *Pantoea*, *Pseudomonas*, *Serratia* and *Burkholderia* are reported to exert biocontrol activities against plant pathogens (Ellis et al., 2001; Notz et al., 2001; Venturi et al., 2004). In addition, some agricultural soils may be less exposed to disease caused by fungi mainly *Fusarium oxysporum*, *Gaeumannomyces graminis*, *Pythium* sp., *Rhizoctonia solani*, and

Streptomyces scabies (Postma et al., 2008). These disease-suppressive soils naturally contain bacteria that exhibit antagonistic capacity, which potentially may control the activity of phytopathogenic fungi against root diseases. Disease-suppressive soils represent a source for beneficial microorganism with biocontrol activity in crops.

The diversity and function of plant associated bacteria is important for the management of agricultural crops and the protection of the environment. Garbeva et al. (2004) reported that many bacterial communities greatly impact on soil ecosystem stability, functioning and resilience to anthropogenic disturbances. Crop-associated bacteria are also involved in the cycling of carbon, nitrogen, phosphorus and sulfur, which are essential nutrients for crop nutrition (Marschner, 2012). Microbial decomposition of organic matter in soil also is important for diverse agriculture practices. For example, microorganisms may mediate availability of most soil fertilizers that are essential for crop growth in organic agriculture systems (Stockdale et al., 2001). In areas where conservation agriculture is practiced, microbial biomass and activity are reported to improve soil quality when reduced tillage and cover crops are implemented (Mbuthia et al., 2015). In addition, Fiers (2012) reported an increase in the diversity of the microbial community when crop rotation was practised, thus suggesting that interactions that occur between microbes and rotations may reduce the impact of diseases and weeds, while increasing crop yields. Although crop associated bacteria play important roles in soil quality and crop growth, microbes are also involved in the degradation of synthetic compounds, such as pesticides and herbicides commonly used in conventional agriculture, thus reducing the impacts of these compounds on the environment (Murphy et al., 2007).

2.2. Bacterial Microbiome Associated with Crops

Bacterial communities associated with crops are not uniformly distributed through plant compartments; and they often exhibit differences in population size and diversity among the rhizosphere, phyllosphere, spermosphere and endosphere compartments. In the current section, characteristics of the bacterial microbiome in each plant compartment, with particular emphasis on bacterial diversity, transmission pathways and the effect on plant growth will be described.

2.2.1. Rhizosphere

The rhizosphere was first defined by Hiltner in 1904 as "the volume of soil around living roots that is influenced by the root activity" (York et al., 2016). The biological processes occurring within the interface between soil and roots have critical implications for crop growth, soil fertility and environment protection (Bakker et al., 2013). Although the rhizosphere is defined as "a volume of soil", the actual delimitation of this compartment is mainly based on the influence that roots exert on the abiotic and biotic components of soil within this interface (York et al., 2016). As a result, depletion zones of water and mineral nutrient due to root uptake, as well as modifications on the physical structure of the soil that are produced by root activity can be considered as the abiotic rhizosphere zones. Conversely, the biotic rhizosphere zones will include communities of microorganisms and animals that are mainly influenced by rhizodeposition; however, abiotic and biotic zones interact and influence many rhizosphere processes (Segal et al., 2008; Aravena et al., 2014; York et al., 2016).

Rhizodeposition is defined as "the release of carbon from the roots into the rhizosphere" (Jones et al., 2009). Carbon compounds originate mainly from the plant photosynthesis and are lost via root cap and border cells, mucilage, root exudates, volatile organic compounds, flow of carbon to root associated symbionts, and death and lysis of root cells (Jones et al., 2009). Main root exudates include: *i*) low molecular weight (amino acids, organic acids, sugars, phenolics and other secondary metabolites) and *ii*) high molecular weight (polysaccharides, proteins, etc.) organic compounds (Badri et al., 2009; el Zahar Haichar et al., 2014). During the rhizodeposition processes, plants export into the soil a large portion of their fixed nutrients, accounting for approximately 11% of net photosynthetically fixed carbon and 10–16% of total plant nitrogen, depending on plant species and age (Jones et al., 2009; Bulgarelli et al., 2013). Elevated rates of rhizodeposition in soils, commonly referred as rhizosphere effect, result in increases of microbial numbers and activities in the rhizosphere when compared to bulk soil (Bekker et al., 2013). In fact, in most bulk soils, lower carbon availability levels limit bacterial growth, whereas in the rhizosphere, organotrophs that are able to obtain their energy from organic carbon released by roots represent most of the rhizosphere bacteria (Bulgarelli et al., 2013; Mendes et al., 2013). Although the rhizosphere effect results in an increase of the total microbial biomass in the rhizosphere, the microbial diversity in this compartment is generally lower than bulk soils (Berendsen et al., 2012; Loeppmann et al., 2016).

Rhizosphere bacteria have been estimated to reach up to 10^{11} microbial cells per gram of fresh root and comprise more than 3×10^4 species (Berendsen et al., 2012). The major bacterial phyla detected in the rhizosphere include: Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Planctomycetes, Proteobacteria and Verrucomicrobia (Bulgarelli et al., 2013; Turner et al., 2013a). The ability to degrade a wide range of labile carbon sources that are released by the roots, fast growth rates and quick adaptation to the rhizosphere, are some factors that explain the predominance of Proteobacteria in the roots of a diverse crop species (Bulgarelli et al., 2013; Peiffer et al., 2013; Chaparro et al., 2014). Frequently, the phylum Actinobacteria is detected in the rhizosphere of crops grown in disease suppressive soils and is been reported to promote plant growth (Tokala et al., 2002; Mendes et al., 2011). Examples of most common genera of rhizosphere bacteria include *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Rhizobia*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Mycobacterium*, *Flavobacter*, *Cellulomonas* and *Micrococcus* (Prashar et al., 2014).

The majority of rhizosphere bacteria exhibit neutral effects on crop growth, mostly due to their ability to metabolize root exudates without affecting plant growth (Raaijmakers et al., 2009). However, many rhizosphere bacteria also exhibit beneficial effects on plant growth. In fact, some of the beneficial effects, as a result of these associations, include: nitrogen-fixation, stimulation of plant growth, increased crop yield, reduced phytopathogen infection, as well as reduced biotic or abiotic plant stress (Compant et al., 2010). Common genera of rhizosphere bacteria frequently reported as PGPB include *Rhizobium*, *Azospirillum*, *Burkholderia*, *Pseudomonas* and *Enterobacter* (Lagos et al., 2015). In contrast, a wide range of pathogenic bacteria are also detected in the rhizosphere. These phytopathogens may exhibit detrimental effects on the host plants mainly due to their inherent ability to produce metabolites with phytotoxic activity (Raaijmakers et al., 2009).

2.2.2. Phyllosphere

The phyllosphere comprises the aerial parts of plants that is dominated by the leaves (Rastogi et al., 2013). As opposed to the rhizosphere, the phyllosphere can be considered a short-lived environment, where colonizing microorganisms must multiply and occupy the newly formed niches while the leaves are still expanding (Vorholt, 2012). Despite these limitations, the phyllosphere hosts a large and diverse microbiota of bacteria, fungi, yeast and archaea (Lindow

and Brandl, 2003). Bacteria are the most abundant inhabitants of the phyllosphere with populations estimates up to 10^8 bacterial cells per cm^2 of leaf surface (Meyer and Leveau, 2012). However, bacterial population size may fluctuate among and within plants of the same species, as well as over short time scales and over the growing season (Ercolani, 1991; Thompson et al., 1993). Fluctuations in the environmental conditions such as diurnal cycle also influence plant metabolism and subsequently the phyllosphere organisms. Thus, oscillations in bacterial populations may be affected by changes in physical and nutritional conditions of the phyllosphere. For instance, the leaf is considered a hostile environment for microbe survival and colonization due to the rapid fluctuation in solar radiation, ambient temperature, humidity, and infrequent availability of nutrients (Lindow and Brandl, 2003; Vorholt, 2012). Low moisture conditions in the phyllosphere may be caused by cuticles that cover plant epidermal cells and protect the leaf against desiccation (Vorholt, 2012). In addition, because of nutrient leaching that may occur from the shoot surfaces, the phyllosphere is an oligotrophic environment (Vorholt, 2012).

The leaf surface is exposed to bio-aerosols consisting of granules colonized by bacteria, fungi, viruses, and/or pollen that are released from terrestrial and marine environments into the atmosphere (Frank et al., 2017). Bacteria are highly abundant in the atmospheric air, reaching concentrations up to 10^6 cells per m^3 of air (Lighthart, 2000). The composition of bacterial communities on plant leaves consists mostly of relatively few bacterial phyla and is usually dominated by Proteobacteria, however, Actinobacteria, Bacteroidetes and Firmicutes also are detected (Bulgarelli et al., 2013). In terms of genera, the most common bacteria inhabiting the phyllosphere include *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, *Bacillus*, *Massilia*, *Arthrobacter*, *Erwinia* and *Pantoea* (Lindow and Brandl, 2003; Bulgarelli et al., 2013).

Most of bacteria in the phyllosphere are commensals, but some leaf associated bacteria also are reported to exhibit beneficial effects on plant growth and health. For instance, some leaf bacterial communities associated with coniferous can degrade excreta released from sucking aphids (Stadler et al., 1998), thus suggesting that bacteria in the phyllosphere are involved in carbon cycling. Bacteria associated with the leaf also are reported to participate in the nitrogen cycle. In fact, Papen et al. (2002) reported that phyllosphere bacteria associated with spruce trees exhibited nitrification activity, whereas Freiberg (1998) isolated cyanobacterial species able to fix atmospheric nitrogen in the leaves of tropical forest trees. In addition, Innerebner et al. (2011) reported that the genus *Sphingomonas* inhibited the foliar pathogen *Pseudomonas syringae* on

Arabidopsis thaliana, thus suggesting that bacteria inhabiting the phyllosphere also may suppress plant disease. Knowledge on ecological aspects of leaf bacteria is important when developing prevention and/or control strategies to mitigate human pathogenic bacteria. For example, *Burkholderia cepacia*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* that are frequently detected in crops are known to be human pathogenic bacteria (Lindow and Brandl, 2003; Brandl, 2006).

2.2.3. Spermosphere

Bacterial colonization of crops begins at seed germination stage and marks the first point of interaction between plants and soil microorganisms. These interactions occur at the soil-seed interface and may result in either beneficial or deleterious effects on plant growth, development and health (Singh et al. 2011). The spermosphere was defined by Nelson (2004) as "a short-lived, rapidly changing, and microbiologically dynamic zone of soil surrounding a germinating seed". Despite the transient nature of the spermosphere, associations initiated within this compartment have important implications for the future establishment of the microbial communities in the rhizosphere, which can significantly influence crop growth (Schiltz et al., 2015).

Seed germination is a series of physiological events that start with seed imbibition (*i.e.*, rapid uptake of water by the dry seed) and are completed when the radicle tip is visible (Weibrecht et al., 2011). During the imbibition process, a quick release of seed exudates from the seed surface into the soil promotes microbial growth resulting in the promotion or inhibition of early seedling growth (Windsor et al., 2000; Yang et al., 2012; Schiltz et al., 2015). Seed exudates consisting of carbon compounds such as of sugars, proteins, amino acids and fatty acids, provide a readily available food source for microbial development in the spermosphere and can modulate the interaction between microbes during early plant growth (Nelson, 2004). However, crop genotype influences the selection of the microbial communities in the spermosphere (Nelson, 2004; Schiltz et al., 2015).

Seed germination may be prevented temporarily due to plant physiological processes, even under apparently optimal environmental conditions, by a trait known as seed dormancy (Taiz and Zeiger, 2002). Several factors including light, temperature, moisture and seed morphology are involved in the control of seed dormancy and/or germination. However, plant hormones also play a role in the regulation of seed dormancy. For example, absicic acid induces dormancy whereas

giberellin promotes seed germination. Additional plant hormones including auxins, brassinosteroids, cytokinins, ethylene and jasmonates also are implicated in the regulation of seed dormancy and germination (Taiz and Zeiger, 2002). Previous studies have reported that spermosphere bacteria increased seed germination by the production of phytohormones, or by inducing seed physiological protection during stress conditions (Dodd et al., 2010; Mastouri et al., 2010). In addition, *Pseudomonas chlororaphis* and *Enterobacter cloacae*, detected in the spermosphere, are reported to control seed-borne diseases, and thus can be classified as potential biocontrol agents (Johnsson et al. 1998; Van Dijk and Nelson, 2000).

2.2.4. Endosphere

The term endophyte is defined as "a microorganism that can be isolated from surface-disinfested plant tissues and cause no visible harm to the host plant" (Hallmann et al., 1997). Usually, endophytes are considered to be non-pathogenic, but they also include latent pathogens that, depending on environmental conditions and/or host genotype, can cause plant disease (Turner et al., 2013a). In addition, commensal endophytes that benefit from metabolites produced by plants, but have no apparent effects on plant growth, are often detected in plant tissues (Hardoim et al., 2015). Bacterial endophytes may be classified as obligate or facultative microorganisms (Hardoim et al., 2008). Obligate endophytes are microbes that depend strictly on the host plant for growth and survival and are unable to proliferate outside the host plant. The transmission of obligate endophytes occurs vertically (*i.e.*, transmitted from the parent plant to its offspring through seeds) or by vectors, rather than originating in the rhizosphere. Some examples of obligate endophytes include: *Xylella fastidiosa*, a bacterial species that is well adapted to colonize plant tissues of citrus without causing harm to the plant (Hardoim et al., 2008) and the N_2-fixing *Anabaena* spp. that establish symbiotic association with the fern *Azolla* spp. (Bright and Bulgheresi, 2010). In contrast, facultative endophytes are free-living in soil, but will colonize plants if conditions are favourable for colonization. Facultative endophytes usually are transmitted horizontally *i.e.*, entering the plant tissues from the environment (Bright and Bulgheresi, 2010).

Most plant associated endophytes are facultative microbes that originate from the soil and are able to penetrate, multiply and persist in the interior of plant tissues (Rosenblueth and Martinez-Romero, 2006). Usually, root colonization by endophytes starts with a bacterial colonization from the rhizosphere. Rhizosphere colonization involves the recognition by the bacteria of specific

compounds in the root exudates, which induce bacteria chemotaxis towards the root (Hardoim et al., 2008). This initial rhizosphere colonization is followed by the attachment of bacteria on the rhizoplane *i.e.*, root surface. Subsequently, endophytes may penetrate root tissues, mostly by accessing lateral root junctions, or by penetrating cracks or wounds that are formed by arthropod or nematode action (Turner et al., 2013a; Martinez-Garcia et al., 2015). Once inside the plant, bacterial endophytes generally colonize intercellular spaces, where the abundance of carbohydrates, amino acids, and inorganic nutrients is plenty (Kandel et al., 2017). Conversely, in endosymbiotic interactions such as the root nodule symbiosis, rhizobia multiply inside the living host cells and/or may be surrounded by a host membrane compartment (Reinhold-Hurek and Hurek, 2011). Following the colonization of root tissues, bacteria may multiply in the root cortex or translocate from roots to aboveground plant organs by accessing the interior of xylem vessels (Compant et al., 2010). In addition, bacteria may also colonize the plant interior by entering the plant stomata, the leaf surface, through flowers, fruits, stems or cotyledons and seeds (Martinez-Garcia et al., 2015). Many of the multiple steps required for bacterial establishment within the plant tissues involves a number of active bacteria physiological traits including: (*i*) production of lipopolysaccharides, (*ii*) activity of flagella and/or pili, (*iii*) secretion of cell wall degrading enzymes and (*iv*) degradation of plant-derived compounds (Compant et al., 2010). In contrast, bacteria termed as passive endophytes, lack active mechanisms used to colonize the host plant; however, they infest internal plant tissues by accessing open wounds along the root hairs (Gaiero et al., 2013).

Estimations of endophyte populations vary within plant tissues and are often influenced by the methodology and media selection (Sturz et al., 2000). Usually, bacterial root endophyte populations range between 10^3 to 10^8 CFU·g^{-1} root fresh weight, depending on plant age and genotype (Turner et al., 2013a). In contrast, in aboveground plant organs, endophyte numbers tend to be lower, with average densities reaching only 10^4 CFU·g^{-1} fresh weight in leaves and stem (McInroy and Kloepper, 1994; Sturz et al., 1997). Previous studies on seed endophytes reported populations densities ranging from 10^1 to 10^7 CFU·g^{-1} of seeds from distinct crops species including beans, grasses, maize, rapeseed, rice, soybean, tomato and wheat (Graner et al., 2003; Rosenblueth et al., 2010; Truyens et al., 2015). Endophytic bacterial communities that associate with crops comprise mostly of Proteobacteria; however, Firmicutes, Actinobacteria and Bacteroidetes are also detected (Reinhold-Hurek and Hurek, 2011). The most commonly found

genera of endophytes consist of *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea* and *Microbacterium* (Santoyo et al., 2016). These genera are also common inhabitants of the rhizosphere, thus suggesting that the plant endosphere may be a subset of the rhizosphere inhabiting bacteria (Germida et al., 1998; Marquez-Santacruz et al., 2010).

Although a diverse group of endophytic bacteria has been reported in crops, their function and/or ecology remain unknown (Hardoim et al., 2012). These endophytic communities are often influenced by factors that include plant growth stage, soil type and agricultural management practices (Hallmann and Berg, 2006). However, microorganisms that are termed "competent endophytes" can successfully colonize plant tissues and promote plant growth, even under adverse conditions (Hardoim et al., 2008). As compared to the associations that occur on the rhizosphere and/or rhizoplane, endophytes may establish closer associations with a host plant (Beattie, 2007). Therefore, the beneficial effects of endophytes to their host plants are in general greater than those of rhizobacteria, which might be greater when the host plant is subjected to biotic or abiotic stress conditions (Compant et al., 2010; Ma et al., 2011).

Seeds are key components in the life cycle of spermatophytes *i.e.*, plants that produce seeds. Seeds can persist for years in a dormant state and, under the suitable environmental conditions, they germinate and develop into a new plant. Although studies on seed-associated microbiota are still scarce, microorganisms inhabiting plant seeds are important to crop growth. Seed endophytes are transmitted from generation to generation and influence the primary composition of plant microbiota (Nelson, 2004). Chee-Sanford et al. (2006) suggested that seed-associated microorganisms may be involved in seed preservation and germination as seed associated bacteria influenced the incidence of seed decay on seed-banks. Usually, the seed microbiome also may include phytopathogens, which potentially may contribute to the dispersal of plant diseases (Truyens et al., 2015). Several authors reported that seed endophytes have the potential to enhance crop growth and health and the mechanisms involved in plant growth promotion may be similar to those exhibited in other plant tissues (Weyens et al., 2009). Commonly reported seed endophytic genera include *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Micrococcus*, *Staphylococcus*, *Pantoea* and *Acinetobacter* (Truyens et al., 2015).

2.3. Factors Affecting the Diversity of Bacterial Communities Associated with Crops

Each plant compartment provides unique ecological niches for bacteria to inhabit these distinct crop tissues. Thus, during the colonization of the crops, bacteria may diversify their functions and adapt to specific conditions that result in the coexistence of diverse bacterial populations (Muller et al., 2016). Therefore, individual bacterial species that associate with crops may be influenced by environmental conditions thereby affecting their growth cycles. These environmental conditions may be classified as modulators (*i.e.*, environmental characteristics that influence the physiology of organisms such as temperature, pH, or salinity) and resources (*i.e.*, physical components of the environment that are used by organisms including nitrogen, energy, territories, or nesting sites) (Van Elsas et al., 2006). The effect of soil properties and crop species on bacterial communities will be described in sections 2.3.1 and 2.3.2.

2.3.1. Soil Physical and Chemical Characteristics

Soil is a natural medium for the growth of terrestrial plants and consist of layers (soil horizons) that are composed of weathered mineral and organic materials, air and water (FAO, 2018). Amongst all terrestrial ecosystems, soils are the most heterogeneous. Soils exhibit an enormous variation on properties and processes that occur within nano- to macroscales (Loeppmann et al., 2016). Hence, due to their highly heterogenous nature, soils have the ability to provide unique habitats for diverse groups of microorganisms, or to affect the physiology of plant roots, which indirecty influence the plant microbiome (Garbeva et al., 2004). Soil bacteria usually tend to aggregate in colonies and/or biofilms, where microbiological processes occur in a much faster and intense rate, compared to the average soil conditions (Ekschmitt et al., 2005; Kuzyakov, 2010). The bacterial activity in these hotspots is modulated by organic matter, moisture, oxygen and nitrogen availability. Additionally, these soil properties also affect denitrification, methanogenesis, nitrification and soil weathering (Loeppmann et al., 2016).

The availability of labile carbon is the main limitation factor that controls bacterial activity in soils (Hodge et al., 2000), thus inputs of labile carbon result in increased activity and soil bacterial biomass (Loeppmann et al., 2016). In fact, fluctuations in the relative abundance of a specific bacterial phyla in soils may be explained by the availability of labile carbon. For example, Fierer et al. (2007) reported that Acidobacteria were more abundant in soils with very low concentrations of labile carbon, whereas the relative abundances β-Proteobacteria and Bacteroidetes were greater

in in soils with high carbon availability. Fierer et al. (2007) also reported that in the rhizosphere of plant species such as corn, Lodgepole pine, ryegrass, oilseed rape and white clover; a lower abundance of Acidobacteria and a higher abundance of Proteobacteria were both related to a high availability of carbon released by the plant roots. As a result, these attributes may allow Proteobacteria and Bacteroidetes to be classified as r-strategists, *i.e.*, bacteria that exhibit fast-growing capacity and higher efficiency in metabolizing readily available carbon sources (Fierer et al., 2007, Peifer et al., 2013). In contrast, the phylum Actinobacteria are commonly classified as K-strategists due to their low growth rates, and high persistency in soils, even under low nutrient availability (Van Elsas et al., 2006). In soil, sources of labile carbon include mostly root exudates, litter, and soil animal feces (Kogel-Knabner, 2002; Jones et al., 2009). Although labile carbon is a main limiting factor for bacterial activity in soil, nitrogen availability also regulates important bacterial process that are involved in the nitrogen turnover including nitrification and denitrification (Strong et al., 1997).

Another important soil characteristic that regulates bacterial diversity is pH, which affects agricultural fields across North and South America, Great Britain, and polar soil ecosystems (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011; Li et al., 2012; Siciliano et al., 2014). Soil pH influences nutrient availability, salinity and organic carbon, thus regulating the physiology of bacterial community in the soil. Soil pH affects bacterial communities directly by imposing physiological limitations for bacteria in the soil, thus modulating competition among species and/or altering the dominance of certain taxa (Lauber et al., 2009). In fact, studies conducted by Sait et al. (2006), Upchurch et al. (2008) and Lauber et al. (2009) confirmed that the relative abundance of Acidobacteria, Actinobacteria, and Bacteroidetes changed across a wide range of soil pH.

Soil texture and spatial connectivity within the soil matrix are also important factors that influence bacterial diversity in soils. In fact, Sessitsch et al. (2001) reported that soil particle size exerts a higher influence on bacterial diversity as compared to other edaphic factors such as pH and organic matter availability. Soil texture influences bacterial communities mainly by modulating water movement and content within the soil matrix (Carson et al., 2010). For instance, Ranjard and Richaume (2001) reported that more than 80% of the soil bacteria are found in micropores of stable soil micro-aggregates. These micropores provide suitable conditions for bacterial growth as they provide water, substrates, ideal gas diffusion rates and protection against

predation. Soil texture and water regime have been reported to modulate the interactions amongst soil bacteria by controlling the connectivity of soil bacteria within the soil matrix (Torsvik and Ovreas, 2002). Most likely, dry soils with low water content will exhibit low connectivity within the soil matrix but will exhibit high bacterial diversity. In contrast, high connectivity soils are frequently highly water saturated and exhibit a much lower bacterial diversity (Torsvik and Ovreas, 2002). The higher diversity observed in soils with low connectivity has been related to a more diverse carbon resources available in these soils, thus resulting in a much wider bacterial niche variation. Soil bacteria also are subjected to considerable seasonal fluctuations in the environment including soil temperature, water content and nutrient availability (Smit et al., 2001).

2.3.2. Crop Species and Plant Development Stages

Plant species and/or cultivars are known to influence the interactions that occur between a host crop and bacteria. During the colonization process, crops may select specific groups of bacteria by actively changing the composition of roots exudates, which in turn may influence the rhizospheric community structure (Garbeva et al., 2004; Jones et al., 2009). Root exudates are reported to act as chemo attractants, which mediate the interaction between plant roots and motile bacteria (el Zahar Haichar et al., 2014). In fact, Phillips et al. (1992), Brencic et al. (2004) and Neal et al. (2012) reported that chemo attractants released in the rhizosphere influenced the root colonization by *Pseudomonas*, *Rhizobium* and *Agrobacterium* in various crops. Root derived specific signalling molecules are also reported to be involved in plant × microbe symbiotic relationships. For example, during the legume-rhizobia symbiosis, the initial bacterial recognition by the plant is mediated by the exudation of molecular signals, which activate the *nod* genes responsible for inducing the nodulation process (Hirsch et al., 2001). Flavonoid compounds are reported as the main molecular signal mediating rhizobia-legume symbiotic interactions; sometimes acting as inducers for certain rhizobia species; sometimes antagonists for other species (Cooper, 2007). Several non-flavonoid molecules exuded by plant roots are demonstrated to induce the expression of nod genes (Cooper, 2007). Plant roots produce, and release, metabolites such as phytoanticipins or phytoalexins, that exhibit antimicrobial activity against crop pathogens, thus modulating the abundance of pathogens in the plant (Bais et al., 2006). Another mechanism that plants may utilize to avoid pathogens including *Erwinia*, *Pseudomonas* and *Agrobacterium* consists on neutralizing the action of virulence molecular signals (Fray, 2002). These signals are responsible for the coordinated actions of bacteria referred as quorum sensing systems. Plant

mechanisms used to counteract virulence signals include the production of mimics, blockers, degrading enzymes, or compounds that inhibit the activity of the pathogens (Fray, 2002; Rasmussen and Givskov, 2006).

Several studies demonstrate that endophytic bacterial communities are a subset of the rhizosphere microbiome that can be selected by crops during the colonization of the root interior (Germida et al., 1998; Bulgarelli et al., 2013; Edwards et al., 2015). Generally, crop selection of endophytic bacteria is dependent on the composition of root exudates in the rhizosphere. However, the plant innate immune system also may contribute to the selection of unique endophytic communities associated to specific crops (Bulgarelli et al., 2013; Muller et al., 2016). Interactions that occur between the plant inmune system and inhabiting bacteria have evolved to distinct responses including not only defense reponses against pathogens, but also mutualist and/or commensals associations between bacteria and host plants (Zipfel, 2014). In fact, Boller and He (2009) suggested that some bacterial endophytes are capable of producing effective mechanisms that can avoid detection by the plant immune system, thus establishing viable cells inside the plant tissues. Other factors modulating the selection of the plant microbiome is the presence of metabolic traits involved in the use of plant-derived substances such as the ability to degrade carbon compounds and the production of plant cell wall components (Muller et al., 2016). Similarly, Ofek-Lalzar et al. (2014) reported that additional bacterial physiological traits may lead to competence of endophytes during the root colonization process. These bacterial traits include: motility, chemotaxis, membrane regulatory systems and secretion systems. Additional factors affecting the selection of specific root bacterial endophytes include root architecture and/or the presence of wounds that may facilitate bacterial access into the host plant roots (Gaiero et al., 2013).

On crop leaves, selection of leaf colonizing bacteria depends on adaptations of the organism to survive in harsh environmental conditions, such as UV radiation, reactive oxygen species, and desiccation (Vorholt, 2012). As a result, some leaf associated bacteria may exhibit traits that alleviate stress including the production of catalase and superoxide dismutase enzymes that are employed during the detoxification of reactive oxygen species (Muller et al., 2016). In addition, bacteria also can produce pigmentation that, together with photolyase activity, avoid UV-induced damage of nucleic acids (Muller et al., 2016). Another mechanism reported that allow the survival of bacteria in harsh environmental conditions of the phyllosphere include bacterial secretion of extracellular polymeric substances and bioactive surfactants. When present on the leaf surface,

these compounds may increase permeability of the plant cuticle, thus improving water availability for epiphytic bacteria (Burch et al., 2014).

Plant growth stages are important factors that can influence the diversity of microbial communities associated with crops (Smalla et al., 2001; Dunfield and Germida, 2003; Farina et al., 2012; De Campos et al., 2013; Copeland et al, 2015; Gdanetz, 2017). Changes that occur in the diversity of bacteria during plant growth stages may be explained by factors including: (*i*) bacterial succession through growth stages as a consequence of root surface increases, which leads to an increased habitat and resources in the rhizosphere, (*ii*), a shift of bacterial communities due to signaling between the host plant and microorganisms that colonize the rhizosphere at earlier stages, and (*iii*) bacterial response to the availability of complex metabolites released by mature plant roots (Gdanetz, 2017). Previous studies have concluded that the presence of certain bacterial groups at specific growth stages can be related to the different ecological strategies of these bacteria and their interaction with the host crop. At vegetative stage for example, the tip of the young roots provides the highest amount of organic carbon that can be rapidly used by r-strategist bacteria (Brimecombe et al., 2000). In contrast, at crop maturity, bacterial communities are reported to be dominated by K-strategists (Chiarini et al., 1998).

2.4. Plant Growth Promotion by Bacterial Endophytes Associated with Crops

The plant microbiome consists on a diverse group of microorganisms that may occupy several plant tissues and establish various types of association with crops. Among these microorganisms, bacteria having a potential to increase crop health and/or yield are commonly referred as plant growth promoting bacteria (PGPB) (Van Elsas et al., 2006). The PGPB represent a unique possibility for improving crop productivity, while improving the effectiveness and sustainability of agricultural ecosystems. In this section, the main mechanisms involved in the plant growth promotion of bacterial endophytes and the perspectives for PGPB application in Saskatchewan agriculture will be addressed.

2.4.1. Mechanisms of Plant Growth Promotion

There are many mechanisms by which endophytic bacteria use to promote plant growth. Some of these bacterial mechanisms are involved in biofertilization, phytostimulation and biocontrol activity detected in crops (Bloemberg and Lugtenberg, 2001; Gaiero et al., 2013). Biofertilization is defined as the increase of nutrient accessibility and/or nutrient supply from the environment to

the plant mediated by bacteria. Some examples of mechanisms that also can improve the nutrient status of plants include: (*i*) biological nitrogen-fixation, nitrification and ammonia oxidation, (Maier and Triplett, 1996; James, 2000; Elbeltagy, 2001; Cocking, 2003; Sessitsch et al., 2012), (*ii*) solubilization of soil minerals such as phosphorus (Oteino et al., 2015; Borah et al., 2017) and (*iii*) the production of siderophores, which can solubilize and sequester iron from soil, thus making it available to plants (Loaces et al., 2011; Sessitsch et al., 2012; Abbamondi et al., 2016).

Phytostimulation is the result of bacteria phytohormone synthesis on plant growth. Phytohormones are substances produced by plants and microorganisms in various plant tissues at certain plant developmental stages (Kazan, 2013; Egamberdieva et al., 2017). Subsequently, low concentrations of phytohormones are distributed through the plant's vascular system thereby exerting physiological functions at remote plant tissues (Neumann et al., 2009). Phytohormones exert important functions in the regulation of plant growth, organ development and reproduction, as well as the plant immune response against phytopathogens (Pieterse et al., 2012). Indole acetic acid (IAA), cytokinins and gibberellins are some examples of phytohormones synthetized by endophytic bacteria (Egamberdieva et al., 2017).

Biological control (biocontrol) activity occurs when bacteria exhibit antagonistic effects against phytopathogens, thus potentially preventing crop diseases. Endophytic bacteria have the ability to control the proliferation of phytopathogens by using direct and/or indirect mechanisms. A direct inhibition of pathogens consists mainly on the microbial synthesis of inhibitory allelochemicals including antibiotics, hydrogen cyanide, volatile compounds, siderophores and antifungal metabolites (Compant et al., 2010; Glick, 2015). Some endophytes are also able to produce degrading enzymes including chitinase, glucanase, protease and lipase; that can hydrolyze the cell walls of many fungal pathogens (Glick, 2015). Conversely, indirect biocontrol mechanisms consist mostly of an induction of plant systemic resistance mechanism that inhibits the activity of a wide range of phytopathogens (Niu et al., 2011; Conrath et al., 2015). Another mechanism involved in biological control refers to mitigation of pathogenic virulence factors such as plant cell degrading enzymes and phytotoxins (Compant et al., 2010). Some of the virulence factors produced by pathogens are regulated by quorum sensing systems known to induce the coordinated response of pathogen populations during the host plant infection process (Miller and Basler, 2001). In contrast, endophytic bacteria may exhibit quorum quenching systems, that can disrupt the

quorum sensing networks of pathogenic bacteria, thus limiting disease severity (Miller and Basler, 2001; Glick, 2015).

Recent studies also have indicated that the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is a key mechanism involved in the promotion of plant growth (Glick, 2014). Due to the relevance of ACC deaminase, the influence of this bacterial mechanism for the plant growth promotion potential of endophytic bacteria associated with crops was investigated in the current thesis. The ACC deaminase is an enzyme that cleaves ACC (the immediate precursor of ethylene in plants) into ammonia and α-ketobutyrate, and it has been detected in soil bacteria and fungi (Glick et al., 2015). The phytohormone ethylene has been detected in all higher plants. Ethylene is involved in the modulation of plant growth and development processes, some of which include mostly shoot and root growth differentiation, adventitious root formation, and fruit ripening (Glick et al., 1998). However, in high concentrations, ethylene may inhibit plant growth or even causing death (Glick et al., 2007). Ethylene production may increase in response to both biotic and abiotic processes (Glick et al., 1998). Thus, by breaking down some of the ACC in plants, bacteria containing the ACC deaminase enzyme can potentially lower plant ethylene levels thereby regulating ethylene inhibition on plant growth (Glick, 2015).

A general model explaining the activity of ACC deaminase was proposed by Glick et al. (1998) (Fig. 2.1). In this model, the ACC deaminase-containing bacteria attach to the surface of either the seed or plant root or colonize the root interior. The release of tryptophan by root exudates induces bacterial synthesis and secretion of the phytohormone IAA. Plants can take up the IAA produced by the root bacteria, which together with IAA produced by plants will affect various plant physiological process. The IAA induces the transcription of plant enzyme ACC synthase, which in turn catalyzes the formation of ACC, thus stimulating the synthesis of ethylene in the plant. Normally, plant ACC is exuded from seeds, roots or leaves (Penrose et al., 2001) and can be taken up by the bacteria associated with these tissues, and subsequently cleaved by ACC deaminase (Penrose and Glick, 2003). The cleavage of exuded ACC by bacterial ACC deaminase decreases the synthesis of ethylene in plant, thus the bacterial cells also act as a sink for ACC. As a result of the association between ACC deaminase producing bacteria and host plant, the inhibitory effects of ethylene produced by biotic and/or abiotic stress conditions may be reduced. In fact, inoculation of plants with ACC deaminase producing bacteria can increase plant growth when plants are exposed to stress conditions including pathogen infection, flooding, drought,

salinity, flower wilting, and high levels of metal and organic contamination (Wang et al., 2000; Mayak et al., 2004; Reed and Glick, 2005; Arshad et al., 2008; Ali et al., 2012; Glick 2012; Li et al., 2013). In addition, the presence of ACC deaminase in rhizobia increased up to 40% the efficiency of nitrogen-fixing nodules when compared to rhizobia strains lacking this enzyme (Ma et al., 2004). However, ACC deaminase activity in rhizobia is much lower when compared to free-living PGPB. As a result, Glick (2014) suggested that free-living PGPB has a potential to protect plants from different abiotic and biotic stresses by lowering ethylene levels through out the plant.

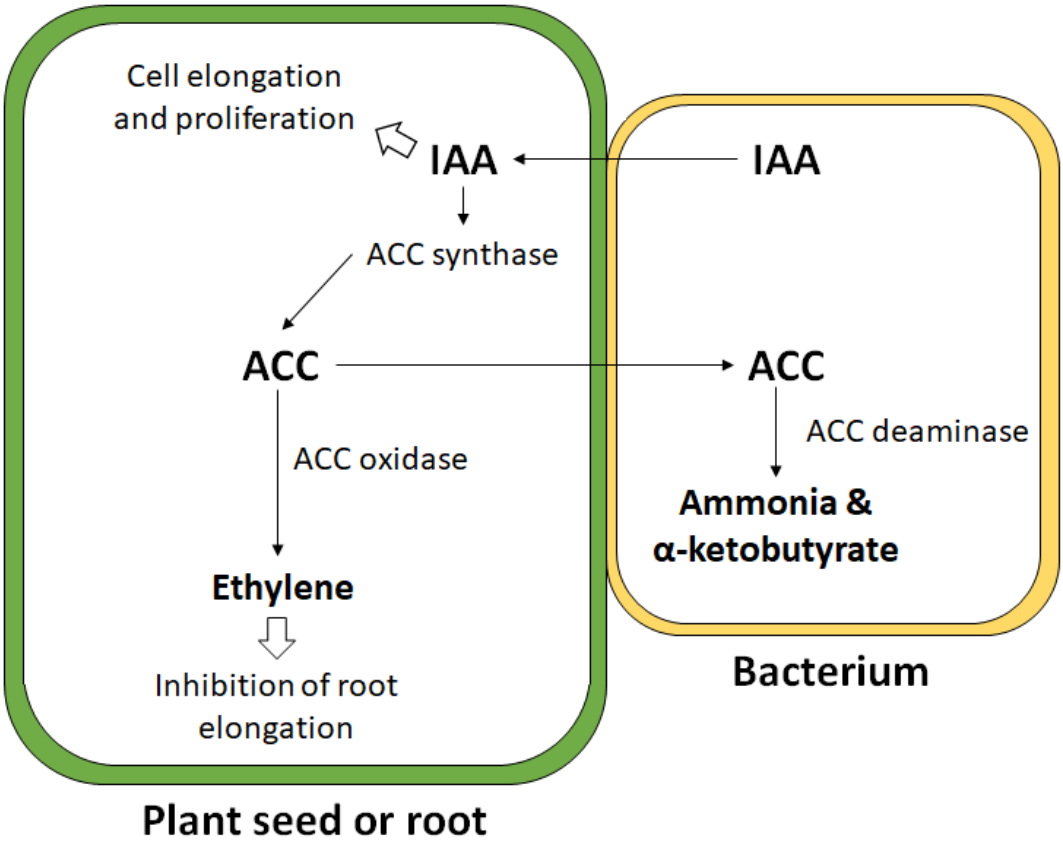

Fig. 2.1. Schematic representation of a general model used to explain the activity of ACC deaminase-containing bacteria associated with plant's seed and/or root (IAA: indole acetic acid, ACC: 1-aminocyclopropane-1-carboxylate) (Glick et al., 1998).

2.4.2. Perspectives for Crop Production in Saskatchewan

Crop production is a significant economic activity in Saskatchewan, mainly contributing to the global exports of grains, oilseeds and pulses (Statistics Canada, 2017). Effective management of crops usually requires intensive application of chemical fertilizers, tillage, irrigation and

pesticides (Foley et al., 2005). However, the implementation of these practices sometimes has negative effects on the long-term soil productivity and the environment. The use of beneficial endophytic bacteria to improve crop development may contribute to sustainable nutrient and pest management systems (Singh et al., 2011). The increasing number of publications demonstrating the ability of endophytic bacteria to promote plant growth and/or health suggest that endophytes have great potential to become effective microbial inoculants that can be used to enhance crop growth (Fig. 2.2). In fact, bacterial inoculants that demonstrate potential to increase crop yields are used extensively in agriculture. Mostly of these inoculants are marketed to enhance nutrient uptake (biofertilizer) and reduce crop loss due to pathogen infections (biocontrol).

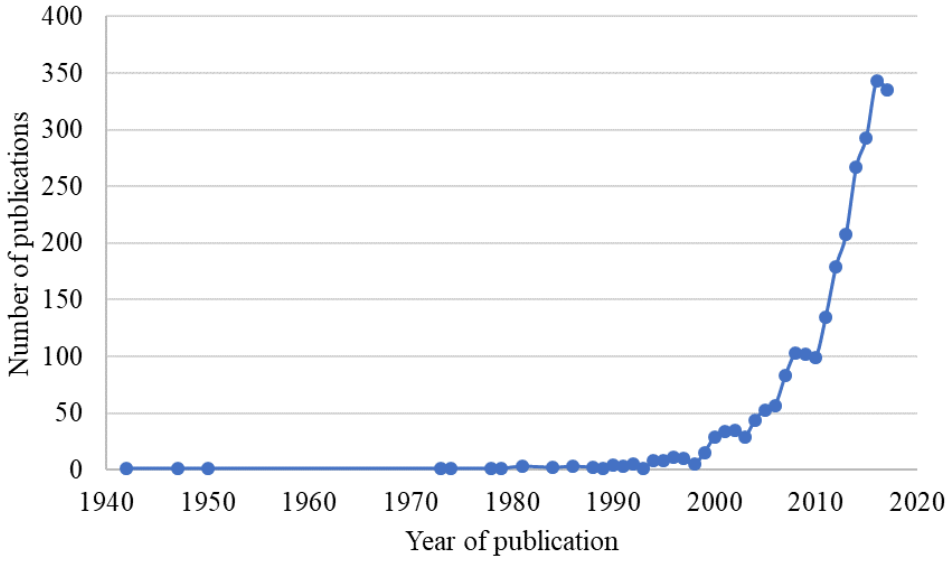

Fig. 2.2. Number of publications from 1940-2017 reporting bacterial endophytes exhibiting plant growth promotion (PubMed database, 2018).

Conventional agricultural practices usually provide nutrients to crops from chemical fertilizers composed of known quantities of nitrogen, phosphorus and potassium. However, approximately 60-90 % of the total applied fertilizer is lost and the remaining 10-40 % is taken up by plants (Bhardwaj et al., 2014). Microbial biofertilizers have a great potential to improve crop nutrition and protect environment (Adesemoye and Kloepper, 2009). The global market for commercial biofertilizers products in 2016 was valued at over US$ 1 million and is expected to exceed US$ 4 million by 2025 (Transparency Market Research, 2018). Currently, cereals and grains are crops with the highest demand for biofertilizers, mostly due to: (*i*) the high consumption of these crop

by increasing world population, (*ii*) the decrease in the arable land area and (*iii*) the considerable amount of biofertilizers needed for proper growth of these crops (Transparency Market Research, 2018). Common bacterial traits contributing to the efficacy of the biofertilizers include biological fixation of nitrogen and solubilization of plant nutrients such as phosphate and potassium. Examples of commercial nitrogen-fixing biofertilizers used to enhance crop growth include strains of *Anabaena, Azotobacter, Clostridium*, *Nostoc* and *Rhizobium*. In addition, commercial phosphate-solubilizing biofertilizers include strains of *Bacillus edaphicus*, *Microbacterium laevaniformans*, *Pantoea agglomerans* and *Pseudomonas putida* (Novozyme 2012; Transparency Market Research, 2018).

Plant diseases are a major problem for crop development worldwide and may cause serious crop losses ranging between 20 and 40 % of the global agricultural productivity. Losses caused by phytopathogens affect not only the food supply, but also public health, economical activities and environment (Savary et al., 2012). Usually, agricultural management practices employ the use chemical products developed for crop protection against phytopathogens. However, long-term applications of chemical products may not be a sustainable solution to control crop disease due to many factors including: development of pathogen resistance, environmental impacts, increasing cost of commercial products, increasing demand for chemical-free food and the incidence of fastidious diseases (Gerhardson, 2002). Biopesticides are known for their ability to avoid the deleterious effects caused by the use of chemicals in agriculture and food production. As a result, biopesticides are considered to be a sustainable alternative for controlling plant diseases (Compant et al., 2005). Biopesticides are defined as "mass-produced, biologically based agents used for the control of plant pests" (Chandler et al., 2008). Some biopesticides include: (*i*) living organisms, (*ii*) naturally occurring substances derived from organisms, and (*iii*) genetically modified plants that express introduced genes that confer protection against pathogens (Copping and Menn, 2000). In 2017, the global market of biopesticide products was valued at over US$ 3 billion and is expected to exceed US$ 9 billion by 2025 (Transparency Market Research, 2018). Among biopesticides, many microorganisms including bacteria, algae, fungi, protozoa and/or viruses, and their associated metabolites, may exhibit effects for crop protection against diseases (Pest Management Regulatory Agency of Canada, 2011). Examples of microbial biopesticide use in Saskatchewan include foliar fungicide applications to control cereal leaf diseases, pulse crop diseases and Late Blight disease in potatoes (Government of Saskatchewan, 2018).

Additional application of endophytic bacteria consists of maintaining sustainable agricultural production under unfavorable environmental conditions, such as drought, extreme soil temperature, soil salinity, as well as pathogens and pests (Glick, 2015). Endophytic bacteria represent a unique possibility for enhancing crop adaptation to stress as they have the advantage of being relatively protected from extreme conditions in the soil (Sturz et al., 2000). Identification of endophytic PGPB for example, may be beneficial for alleviating plant stress conditions in some agricultural areas in southern Saskatchewan where salinity and/or drought are severe crop yield limiting factors (AAFC, 2017). In fact, endophytic bacteria are reported to contribute to plant adaptation under drought conditions due to their ability to accumulate and translocate assimilates, to maintain cell wall elasticity, to regulate osmotic functions and produce ACC deaminase (Glick et al., 1998). In addition, Visser-Tenyenhuis et al. (1994), Nowak et al. (1995) and Senthilkumar et al. (2008) reported that endophytic bacteria introduced in tissue culture propagules *in vitro* of potato, rice and soybean allowed the adaptation of these crops to environmental stresses.

Formulation of bacterial inoculants requires knowledge on the ability of bacteria to colonize, multiply and persist in the host crop, as well as their ability to adapt to the various biotic and abiotic conditions commonly prevalent in agricultural fields (Finkel et al., 2017). Usually, this process is a multistep procedure that may include: bacteria isolation and identification, laboratory *in vitro* tests, and subsequent microcosm, greenhouse and field studies (Fig. 2.3). After an initial set of experiments set-up to obtain the microbe/mechanism in a laboratory, it is important to assess the inoculant on the field as many bacterial strains can stimulate plant growth under laboratory conditions but fail to exhibit the predicted impact on crop growth when applied at a field-scale (Bacilio et al., 2017).

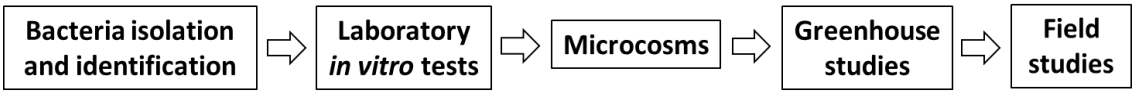

Fig. 2.3. Recommended protocol to formulate bacterial inoculants used in agricultural crops (Pest Management Regulatory Agency, 2001).

Isolation and identification of beneficial endophytic bacteria often are conducted using culture dependent techniques. Usually, cultivation of endophytic bacteria involves surface disinfection of plant tissues, maceration and/or disruption followed plating the resulting suspension onto an appropriate growth medium (Le Cocq et al., 2017). However, due to the fact that only a small fraction of bacteria is culturable, the culture dependent method has a limited ability to isolate diverse groups of endophytic communities that are commonly associated with crops (Kent and Triplett, 2002). Culture dependent method still provides important information that is used during the discovery of potential PGPB inoculants, and to assess possible mechanisms involved in plant × bacteria interactions (Finkel et al., 2017). However, typical plant growth promoting mechanisms that are frequently tested using a culture dependent method, often fail to produce an effect on crop growth, thus suggesting that novel mechanisms are still to be determined (Finkel et al., 2017). Recently, the use of high-throughput DNA sequencing techniques has provided a better understanding of the phylogenetic diversity of plant microbiomes and the functional role of bacteria inhabiting crops (Bulgarelli et al., 2013).

In vitro tests conducted in a laboratory are intended to determine the optimum range of physical and chemical factors required for the growth and survival of a bacteria agent in the inoculant. These factors are important for the establishment of technical characteristics of inoculant's formulations such as inoculation techniques (soil or seed application), mass culture production, bulk sterilization, seed coating, shelf-life, and moisture (Bashan et al., 2014). *In vitro* studies using gnotobiotic conditions, allow to determine the effect of individual and/or strain combinations on plant growth. For example, the use of root elongation assays on agar plates, agar slants or plant growth pouches allow testing bacterial strains for root growth promotion (Penrose and Glick, 2003; Jones et al., 2013). In addition, gnotobiotic studies also can be used to assess potential beneficial effects of bacteria on seed germination (Rajjou et al., 2012). Methods commonly used to study plant bacterization, survival and inoculation effects include seed germination tests. Viable crop seeds exhibit advantages that make them sensitive and quick model systems to test plant growth promotion effect of inoculants. The rapid changes in metabolism, nutrient transport and cell division cause germinating seeds to be highly sensitive to changes in the environmental conditions and thus susceptible to bacterial colonization (Wang, 1991; O'Callaghan, 2016). In addition, seed application is an efficient method for inoculation of crops in field conditions, because after seed germination bacteria will colonize and influence the roots

and rhizosphere (O'Callaghan, 2016). As a result, germination and root elongation tests are relatively simple and efficient ways to test inoculant efficacy to protect the crop (Wang et al., 2001).

Microcosm studies consist of small ecosystems that are simulated in containers exposed in controlled environmental conditions. Microcosms allow replication of treatments and simplify the manipulation of numerous parameters under investigation (Fraser and Keddy, 1997). As a result, microcosms represent a useful tool to test ecological theories by simplifying the complexities of the natural environment (Vidican and Sandor, 2015). Commonly microcosm systems include: plant growth in containers or pots filled with substrates (soil, perlite, vermiculite, sand or calcined clay), hydroponic systems and Leonard jars (Trung and Yoshida, 1983; Fraser et al., 2004; Jones et al., 2013; Lee and Lee, 2015). Conversely, greenhouse studies are conducted under simulated outdoor conditions, to obtain a better understanding of the effect of the inoculant on crop growth, thus these studies can be considered as a link between a laboratory test and field trails (Asea et al., 2005; Farias Neto et al., 2008; Kuan et al., 2016). Field studies evaluate the ultimate performance of the inoculant on plant yield and the bacteria persistence (Chibeba et al., 2015; Fukami et al., 2016; Kumar et al., 2017).

3. THE BACTERIAL MICROBIOMES ASSOCIATED WITH THE RHIZOSPHERE AND ROOT INTERIOR OF CROPS IN SASKATCHEWAN, CANADA

3.1. Preface

The bacterial microbiome influences crop growth and health through interactions between the host plant, bacteria and the soil environment. Despite the importance of rhizosphere and root endophytic bacteria to crop production, little is known about these potential beneficial associations in Saskatchewan field crops. The goal of this Chapter was to investigate the diversity of root and rhizosphere bacteria associated with canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown in Saskatchewan agricultural soils using culture dependent and independent techniques. A total of 298 endophytic bacteria strains were isolated in this study and assessed further for plant growth promotion potential in Chapter 5.

3.2. Abstract

Root associated bacteria are an important component of the plant-microbiome and influence growth and productivity of crops in agricultural ecosystems. Because of the potential benefits of bacteria for sustainable agriculture, it is important to investigate their diversity in different plant species and how edaphic factors influence this relationship. This study assessed bacterial communities associated with the rhizosphere and root interior of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown at four agricultural fields in Saskatchewan. High-throughput sequencing and Denaturing Gradient Gel Electrophoresis (DGGE) analyses of 16S rRNA amplicons from bacterial communities suggested a selection of root endophytic bacteria from the rhizosphere. Proteobacteria, Actinobacteria and Bacteroidetes were the dominant phyla in the root interior, whereas Gemmatimonadetes and Firmicutes were only present in the rhizosphere soil. The genera *Pseudomonas* and *Stenotrophomonas* were predominant in the rhizosphere and root interior of all crops, suggesting a generalist distribution of these bacteria. However, other genera including *Xanthomonas*, *Arthrobacter*, *Streptomyces*, *Acinetobacter*, *Variovorax* and *Rhizobium* were dominant in the root interior of specific crops. Relative abundance of specific bacterial groups in the rhizosphere, as well as bacterial Phospholipid Fatty Acids (PLFA) in the bulk soil, were significantly correlated with soil pH, silt and organic matter contents. However, a lack of correlation between soil properties and most abundant bacterial endophytes was observed, thus suggesting that soil characteristics may not influence bacterial communities within the plant roots. The isolation and identification of culturable bacteria (n=298) using culture dependent methods also revealed that crops selected for specific endophytic bacteria.

3.3. Introduction

Agricultural production is crucial for supplying food to a human population which is predicted to grow over 7 billion in the future (FAO, IFAD, UNICEF, WFP and WHO, 2017). Improving crop yield while optimizing the use of fertilizers, water and pesticides is a continuous challenge in agricultural production. In this context, interactions between crops and microbes are important for the improvement of plant health, nutrient uptake, disease control and stress resistance (Paul, 2014). Many microorganisms including bacteria exhibit versatile physiological mechanisms that potentially may promote plant growth such as nitrogen fixation, phosphate solubilization, production of phytohormones, enzymes and antibiotics (Van Elsas et al., 2006). Some of these bacterial mechanisms can be critical for the establishment and development of crops in agricultural fields. For instance, symbiotic associations that occur between legumes and rhizobia have been extensively used in agriculture. It has been estimated that bacteria alone provide 2.95 Tg and 18.5 Tg of the annual input of fixed nitrogen for pulses and oilseed legumes, respectively (Herridge et al., 2008). Besides their potential for improving crop growth and nutrition, bacteria may also reduce deleterious effect of fertilizer application as well as mitigate the emissions of greenhouse gases (Richardson et al., 2009).

Due to its interaction with soil, the root and its microbiome are perhaps the most important components influencing crop growth. Most of the bacteria associated with crops derive from the bulk agricultural soil (Turner et al., 2013a), however, to exert beneficial effects on crops they need to be in a close relationship with the host plant (Vessey, 2012). For example, the release of root exudates by the crops is an important source of substrates that can be available to a wide group of microorganisms in the rhizosphere. These substrates give rise to a complex and diverse microbial network in the rhizosphere compared to the surrounded bulk soil (Zhang et al., 2017). Some microbes termed as endophytes, colonize plant interior organs during all or part of their life cycle without causing disease to their host (Wilson, 1995). These microorganisms may be transferred among generations by seeds or plant propagules, or penetrate internal tissues from aboveground plant surfaces or from the rhizosphere soil (Frank et al., 2017). Root endophytes may colonize the root intercellular spaces of the host plant by colonizing the cracks formed in lateral root junctions, through the leaf stomata or by wounds caused by microbial or nematode phytopathogens (Hardoim et al., 2008). In the past, a healthy plant was considered to be free of microorganisms, whereas any endophytic bacteria were assumed to be phytopathogenic (Compant et al., 2010). However, recent

studies have demostrated numerous benefits of root bacterial endophytes in agricultural crops. For example, certan endophytic bacteria are able to fix atmospheric nitrogen in association with crops such as rice, sugarcane and canola (Elbeltagy et al., 2001; Boddey et al., 2003; Puri et al., 2016). Other authors reported that endophytic bacteria also can ameliorate abiotic stress condictions such as salinity stress on tomate and drought on maize (Ali et al., 2014; Sandhya et al., 2017). Endophytes can also control crop diseases such as wheat head blight, Verticillium wilts in olive and damping-off of tomato seedlings caused by *Rhizoctonia solani* (Goudjal et al., 2014; Martínez-García et al., 2015; Herrera et al., 2016).

The diversity and abundance of plant associated bacteria may be affected by biotic and abiotic factors (Sturz et al., 2000). Among all factors, crop type and soil characteristics play an important role on the composition of endophytic bacteria within the root. Soil properties have a direct influence on microbial community structure by providing a specific habitat selecting distinct group of microorganisms, or indirectly, by affecting the physiology of plant root (Garbeva et al., 2004). However, several authors reported that plant species and/or cultivar are important factors for establishment of root microbiome (Germida et al., 1998; el Zahar Haichar et al., 2008; Ofek-Lalzar et al., 2014). Crop species may select bacteria due to differences in composition of root exudates that are available to the bacterial communities in the rhizosphere (el Zahar Haichar et al., 2008).

Canada is a global leader in food exports, thus Canadian agriculture has a significant influence on the global food market. Within Canada, Saskatchewan agriculture accounted for more than 40 % of total field crop acreage in 2017 (Statistics Canada, 2017). Canola and wheat are the two largest crops in terms of planted area. Additionally, pulse crop production is an important agronomical activity in Saskatchewan accounting for around 80 % of the total pulse area within Canada (Statistics Canada, 2017). Several studies on crop-associated bacteria in Saskatchewan crops assessed the diversity of the rhizosphere and endophytic bacteria (Dunfield and Germida, 2001; Germida et al., 1998; Misko and Germida, 2002; Siciliano and Germida, 1999); the isolation and characterization of plant growth promoting bacteria (de Freitas and Germida, 1992; de Freitas et al., 1997; Grayston and Germida, 1990) and biocontrol bacteria (de Freitas and Germida, 1991). Other studies in Saskatchewan also analyzed the effect of different agronomic factors on bacterial communities such as crop rotation, tillage, herbicides, fungicides and manure application (Hamel et al., 2010; Helgason et al., 2010; Sprout et al., 1991; Sheng et al., 2012; Dunfield et al., 2000; de Freitas et al., 2003). Improving the current understanding of the bacterial microbiome associated

with crops is important to effectively use these microorganisms in sustainable agriculture and exploit their potential for plant growth promotion in Saskatchewan agriculture.

In this study was hypothesized that plants growing in different agricultural soils associate with unique populations of rhizosphere and root bacterial endophytes. The main objective was to determine the diversity of bacteria associated with the rhizosphere and root interior of wheat, pea, lentil and canola plants grown in different agricultural soils in Saskatchewan using culture-dependent and independent methods.

3.4. Materials and Methods

3.4.1. Sampling and Processing

Canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) were collected from farmers fields during the 2013 and 2014 growing seasons. Plants were harvested at the flowering stage from sites in Central Butte, Stewart Valley, Saskatoon, and Melfort in Saskatchewan (Fig. A.1, Appendix A). Information on GPS coordinates, precipitation, temperature and crop cultivars sampled on each location are provided in Table 3.1. Each crop was rotated within each location from 2013 and 2014. Four samples, consisting of 4 to 6 plants and adhering soil were taken from rows in each field by excavating at 10 cm depth (Fig. A.1, Appendix A). Plants and roots were collected 10 m apart in each row. Samples were kept in plastic bags, stored in ice coolers and transported to the laboratory.

3.4.2. Analysis of Soil Physical and Chemical Properties

Bulk soil was sieved (<2mm) and stored at -20°C for physical and chemical characterization. Soil samples were sent to ALS Environmental Laboratory (Saskatoon, Saskatchewan) for basic soil analysis (Table 3.2). Soil pH was measured in a 2:1 soil: water slurry. Soil organic matter (OM), was determined using the dry-ash method (McKeague, 1978). Soil available nitrate was determined according to Laverty and Bollo-Kamara (1988). Available phosphorus and potassium in soil were determined using a modified Kelowna method (Qian et al., 1994). Available sulfate was measured by a calcium chloride extraction (McKeague, 1978). The particle size was analyzed using the Mini-Pipet Method (Burt, 2009). Available nitrate, phosphorus, potassium and sulfate in soil were expressed in $mg \cdot kg^{-1}$ of soil, whereas particle size and organic matter content were expressed in %.

Table 3.1. GPS coordinates, soil type, mean precipitation, mean temperature and crop cultivars collected at Saskatchewan agricultural fields.

Location	Central Butte	Stewart Valley	Saskatoon	Melfort
	(50°43'N, 106°25'W)	(50°37'N, 107°39'W)	(52°10'N, 106°30'W)	(52°49'N, 104°36'W)
Soil order [1]	Brown Chernozem	Dark Brown Chernozem		Black Chernozem
Mean precipitation [2] (mm)				
2013	40.4	63.3	56.1	70.9
2014	61.8	103.0	52.6	88.0
Mean temperature [2] (°C)				
2013	17.1	16.7	17.3	16.5
2014	16.8	15.9	16.8	16.4
Crop cultivars				
Canola				
2013	Invigor L150	Clearfield	Invigor L150	Canterra1990
2014	Invigor L150	Clearfield	Invigor L150	Canterra1990
Wheat				
2013	Waskeda	-	CDC Utmost	Unity
2014	Waskeda	-	CDC Utmost	Unity
Pea				
2013	CDC Meadow	CDC Meadow	CDC Meadow	CDC Meadow
2014	CDC Meadow	-	CDC Meadow	CDC Meadow
Lentil				
2013	CDC Maxim	CDC Inpress	IBC-605	-

(1) Following the Canadian System of Soil Classification (Soil Classification Working Group, 1998).

(2) Mean precipitation and temperature are the average of monthly values from June-August collected by Environment Canada weather stations situated at Elbow (near Central Butte), Stewart Valley, Saskatoon and Melfort, in Saskatchewan.

Table 3.2. Physical and chemical properties of soil samples collected at Saskatchewan agricultural fields in Central Butte, Stewart Valley, Saskatoon and Melfort.

Location	Crop	Year	pH	sand	silt	clay	OM	Available NO_3^-	Available SO_4^{2-}	Available PO_4^{3-}	Available K^+
				(%)				($mg \cdot kg^{-1}$ soil)			
Central Butte	Canola	2013	7.6	42	40	18	3.8	25.3	10.1	73.9	603
		2014	8.0	51	38	11	3.4	12.4	6.8	23.5	586
	Wheat	2013	8.1	68	22	9	2.1	3.0	6.9	39.0	367
		2014	7.6	65	25	10	3.1	5.5	6.6	28.6	584
	Pea	2013	6.9	44	41	16	3.3	12.5	8.5	34.5	435
		2014	7.9	43	46	11	4.3	6.8	4.2	47.3	1640
	Lentil	2013	6.9	46	38	17	3.4	14.2	14.2	34.9	373
Stewart Valley	Canola	2013	7.5	12	35	54	4.7	5.0	9.5	30.1	525
		2014	7.4	9	35	56	3.1	6.3	13.0	24.1	748
	Pea	2013	7.5	11	31	58	4.4	5.2	8.9	10.0	331
	Lentil	2013	7.6	10	30	59	4.6	8.1	12.3	32.2	525
Saskatoon	Canola	2013	7.8	15	43	43	4.5	19.5	13.8	26.0	389
		2014	6.9	17	44	39	5.6	22.0	3.8	48.5	660
	Wheat	2013	6.8	27	50	24	4.9	20.1	12.5	60.2	510
		2014	7.4	17	39	44	4.3	14.0	3.3	42.6	566
	Pea	2013	7.7	12	41	48	5.0	23.4	15.8	45.9	519
		2014	7.2	23	46	31	5.2	24.6	4.4	33.5	505
	Lentil	2013	7.2	31	41	28	4.1	30.4	11.5	16.2	358
Melfort	Canola	2013	6.5	11	55	34	11.5	17.0	11.4	54.5	425
		2014	6.5	9	61	30	11.8	17.9	5.2	79.7	838
	Wheat	2013	6.5	11	59	30	10.0	44.0	11.4	20.1	384
		2014	6.1	15	56	30	14.6	15.9	7.1	10.7	624
	Pea	2013	6.2	12	54	34	10.4	25.1	7.6	30.5	476
		2014	6.3	7	72	21	11.4	14.5	4.3	32.1	303

3.4.3. Phospholipid Fatty Acids (PLFA) Analysis

The PLFAs were extracted and analyzed to assess relative abundance and community structure of microbial groups in bulk soils as described by Helgason et al. (2010). Briefly, fatty acids were extracted from 4.0 g of lyophilized ground soil. Fatty acids were separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON). Then, phospholipids were methylated and the resulting fatty acid methyl esters analyzed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a 25 m Ultra-2 column (J&W Scientific). Peaks were identified using fatty acid standards and MIDI^{TM} identification software (MIDI Inc., Newark, DE) and quantified based on the addition of a known concentration of the internal standard methyl nonadecanoate (19:0).

Specific biomarkers were used to assess the relative abundance of bacterial functional groups in the soil. Fungal and bacterial biomass in the soils were assessed using biomarker 18:2 ω6,9 and the sum of 13 biomarkers (i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0), respectively (Baath and Anderson, 2003). Biomarkers representing Gram positive bacteria (G+) included i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 (Hedrick et al., 2005), whereas biomarkers representing Gram negative bacteria (G-) included 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0 (Macdonald et al., 2004). Abundance of arbuscular mycorrhizal fungi (AMF) were evaluated using the PLFA biomarker 16:1ω5c (Olsson, 1999). Biomarkers abundance were calculated based on the peak area detected for each fatty acid, relative to that of a known quantity of the internal standard. PLFA values were reported as absolute biomass (nmol·g^{-1} soil) and relative abundance (mol%) based on soil air-dry mass (g).

3.4.4. Bacterial Isolation and Identification using Culture Dependent Methods

Rhizosphere and endophytic bacteria were isolated using the protocol described by Siciliano and Germida (1999). To isolate bacteria from the rhizosphere, roots (2 g) with adhering soil were placed in a 500 mL Erlenmeyer flask containing 200 mL of sterile phosphate-buffered saline (PBS; 1.2 g·L^{-1} Na_2HPO_4, 0.18 g·L^{-1} NaH_2PO_4, 8.5 g·L^{-1} NaCl; pH 7.6) and placed on a rotary shaker (150 rpm) at 22°C for 25 min. This solution was serially diluted and 0.1 mL of the 10^{-5} to 10^{-9} dilutions spread plated (n=3) onto 1/10th strength tryptone soy (1/10 TSA) solidified with 1.5% agar. Cycloheximide (50 mg·L^{-1}) was used as a fungicide to prevent fungal growth in the media.

Inoculated plates were incubated at 28ºC and colony-forming units (CFU) determined after 72 h of incubation.

Endophytic bacteria were recovered after removing rhizosphere organisms. Roots (1 g) were transferred into a 300 mL Erlenmeyer flask containing 100 mL NaClO (1.05% $v \cdot v^{-1}$) in sterile PBS and placed on a rotary shaker (150 rpm) at 28°C for 15 min. Roots were rinsed 10 times with 100 mL sterile of tap water and 0.1 mL of the final wash spread onto agar plates of 1/10 TSA to check for contamination. Surface sterilized roots (1 g) were macerated with a sterile mortar and pestle containing 10 mL sterile PBS. Roots nodules from lentil and pea plants were removed after surface disinfection. The root/PBS mixture was serially diluted in sterile PBS and 0.1 mL of the 10^{-3} to 10^{-7} dilutions spread onto 1/10 TSA plates (n=3). Inoculated plates were incubated at 28ºC and CFU counted after 72 h of incubation. Morphologically distinct colonies were isolated by streaking twice on 1/10 TSA. Purified endophytic strains were stored in a 1:1 mixture ($v \cdot v^{-1}$) of 1/10 Tripticase soy broth (TSB) and glycerol at -80°C.

Identification of bacterial endophytes was performed using molecular techniques. Individual colonies were grown in 1/10 TSB at 28ºC on a gyratory shaker (150 rpm) for 72 h. The DNA extraction was performed using the UltraClean Microbial DNA isolation Kit (MO BIO Laboratories, Inc.) following the manufacture's protocols. Extracted DNA was used as template for PCR. The amplification of 16S rRNA gene was performed using the primer pair EUB338 (5'-ACT CCT ACG GGA GGC AGC AG-3') and EUB518 (5'-ATT ACC GCG GCT GCT GG-3') (Lane, 1991; Muyzer et al., 1993). PCRs were performed in a reaction volume of 20 µL consisting of 1 µL of DNA extract, 1 µL each primer (25 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.25 µL BSA (10 $mg \cdot mL^{-1}$) (Bovine serum albumin, Amersham Biosciences, Mississauga, ON, Canada), 10 µL of Hot Star Master Mix and 6.75 µL of RNase-free water (Qiagen, Toronto, Ontario, Canada). Amplifications were accomplished for 30 cycles of 1 min denaturing at 94ºC, 30 sec annealing at 53ºC and 1 min extension at 72ºC. Amplified fragments sized about 200 bp were confirmed by electrophoresis on 1.5% agarose gels in 1×TBE buffer containing the SYBR™ safe DNA gel stain (Invitrogen) and visualized with UV light. Purified PCR amplicons were sequenced by Macrogen Inc. (Seoul-Rep. of Korea). Bacteria were identified by comparison of DNA sequences in GenBank databases using the BLAST algorithm (Altchul et al., 1997).

3.4.5. Survey of Rhizosphere and Root Endophytic Bacterial Communities

Plant roots (2 g) with adhering soil were placed in a 500 mL Erlenmeyer flask containing 200 mL of sterile PBS and placed on a rotary shaker (150 rpm) at 28°C for 25 min. Then, resulting soil slurry was transferred to 50 mL Falcon tubes and centrifuged (2000 × *g* for 5 minutes). The supernatant containing PBS buffer was discarded and the rhizosphere soil stored at -80°C for DNA extraction (Dunfield and Germida, 2003). After removing rhizosphere soil, roots were transferred into a 300 mL Erlenmeyer flask containing 100 mL NaClO (1.05% $v \cdot v^{-1}$) in sterile PBS and placed on a rotary shaker (150 rpm) at 22°C for 15 min. Roots were rinsed 10 times with 100 mL sterile tap and 0.1 mL of the final wash spread onto agar plates of 1/10 TSA to check for contamination. (Siciliano and Germida, 1999). Root nodules from lentil and pea plants were removed after surface disinfection. Sterile roots were chopped aseptically into 2-3 mm and stored in sterile vials at -80°C for DNA extraction. Root nodules from lentil and pea plants were removed prior to DNA extraction.

3.4.6. Analysis of Bacterial Communities using Denaturing Gradient Gel Electrophoresis (DGGE)

Community structure of the soil and root bacteria was examined by DGGE. Total genomic DNA was extracted from bulk and rhizosphere soil and surface sterilized roots using a soil and plant DNA extraction kit (MO BIO Laboratories, Inc.), respectively. The amplification of 16S rRNA gene was performed using the primer pair U341 (with GC-clamp) (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG_CCTACGGGAGGC AGC AG-3') and U758 (5'-CTACCAGGGTATCTAATCC-3') (Phillips et al., 2006). PCRs were performed in a reaction volume of 50 µL consisting of 1 µL of DNA extract, 1 µL each primer (25 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.63 µL BSA (10 $mg \cdot mL^{-1}$) (Bovine serum albumin, Amersham Biosciences, Mississauga, ON, Canada), 25 µL of Hot Star Master Mix and 21.37 µL of RNase-free water (Qiagen, Toronto, Ontario, Canada). Amplifications were performed for 10 cycles of 1 min denaturing at 94ºC, 1 min annealing at 65-55ºC and 1 min extension at 72ºC. This was followed by 20 cycles using an annealing temperature of 55ºC. Touchdown PCR was used in the annealing step to minimize nonspecific priming. Amplification fragments sized about 417 bp were confirmed by electrophoresis on 1.5% agarose gels in 1×TBE buffer containing the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada).

The PCR amplification products were analyzed using DGGE for the visualization of DNA bands representing dominant bacterial species (Muyzer et al., 1993). Briefly, amplicon aliquots were loaded onto an 8% acrylamide gel with a 40-60% denaturing gradient. Electrophoresis was performed for 16h at 80V and 60°C, and the resulting gels were stained with the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada). Random dominant bands were excised from the gel using a sterile scalpel, vortexed briefly in 60 µL of TE buffer, eluted for 30 min at 37ºC and centrifuged at 10000 × *g* for 1 min at room temperature. Then, DNA was re-amplified using the primers U341 (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG-3') and U758 (5'-CTACCAGGGTATCTAATCC-3') (Phillips et al., 2006). PCRs were performed in a reaction volume of 50 µL consisting of 1 µL of DNA extract, 1 µL each primer (25 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.63 µL BSA (10 $mg{\cdot}mL^{-1}$) (Bovine serum albumin, Amersham Biosciences, Mississauga, ON, Canada), 25 µL of Hot Star Master Mix and 21.37 µL of RNase-free water (Qiagen, Toronto, Ontario, Canada). Amplifications were performed for 25 cycles of 1 min denaturing at 94ºC, 1 min annealing at 64ºC and 1 min extension at 72ºC. PCR Amplified fragments were run on 1.5% agarose gels in 1×TBE buffer containing the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada). Finally, PCR amplicons were sequenced by Macrogen Inc. (Seoul, Rep. of Korea). Bacteria were identified by comparison of DNA sequences in GenBank databases using the BLAST algorithm (Altchul et al., 1997).

3.4.7. Analysis of Bacterial Communities using 16S rRNA High-Throughput Sequencing

Total genomic DNA was extracted from rhizosphere soil and surface sterilized roots using a soil and plant DNA extraction kit (MO BIO Laboratories, Inc.), respectively. DNA yield was quantified using Qubit DNA HS Assay Kit (Thermo Fisher Scientific). DNA samples were submitted for high-throughput sequencing to the Génome Québec Innovation Centre, McGill University using Illumina technology. PCR amplifications were conducted using the primers 520F (5`-AGCAGCCGCGGTAAT-3`) and 799R2 (5`-CAGGGTATCTAATCCTGTT-3`) that amplifies the V4 region of the 16S rRNA gene (Edwards et al., 2007). Sample libraries were prepared according to the MiSeq reagent kit preparation guide (Illumina, San Diego, CA), and the sequencing protocol from Caporaso et al. (2010b).

3.4.8. Bioinformatics and Statistical Analyses

Sequences derived from rhizosphere and endophytic root bacteria using high-throughput Illumina technology were analyzed using Mothur version 1.34.3 (Kozich et al., 2013). The standard operating procedure included the generation of contigs from the combination of forward and reverse reads and the removal of sequence errors and chimeras. Sequences from chloroplasts, archaea, eukaryotic organisms were also removed. Taxonomic classification was done with naive Bayesian classifier using SILVA database. Operational taxonomic units (OTU) numbers were calculated at a distance 0.03 (97% similarity). Relative abundance of a bacterial taxa in a sample was calculated as the percentage of sequence reads belonging to the bacterial taxa in relation to the total number of reads in a sample. Rarefaction curves values, Simpson's reciprocal (1/D) diversity and Chao 1 richness were generated using Mothur software at OTU cutoffs of 0.03 distance units using the number of observed OTUs. The influence of locations and crops on the bacteria OTU distribution was analyzed by principal coordinate analysis (PCoA) using QIIME (Quantitative Insights Into Microbial Ecology 1.9.1) (Caporaso et al., 2010). Heatmaps were conducted using the VEGAN package (version 2.0–7) in R version 2.15.2 (R Core team, 2012). The sequence data can be accessed in NCBI under Genome Project ID 510213 (accession PRJNA510213).

During DGGE gel analysis, band detection and cluster analysis were performed using Bionumerics version 5.1 (Copyright © 1998 Applied Maths, Austin, TX). Detection of DGGE bands in the gel pictures was performed using a minimum profiling, position tolerance and optimization of 5%, 1.5% and 2%, respectively. Densitometric curves were used to perform band matching, creating a binary presence-absence matrix (Boon et al., 2002; Peixoto et al., 2006). The binary matrix was subsequently subjected to non-metric multidimensional scaling (NMDS) analysis. Cluster analysis was performed using the Pearson correlation coefficient based on densitometric curves and the Ward linkage method.

The influence of locations and crops on the bacterial community profile based on DGGE and PLFA was analyzed by non-metric multidimensional scaling (NMDS) using PCOrd software (McCune and Grace, 2002) with the Autopilot Slow and Thorough analysis options. The statistical significance of the final solution was determined by comparing the final stress values among the best solution for each axis using the Monte Carlo test. Final stress consists of the square root of

the normalized squared discrepancies between interpoint distances in the NMDS plot and the smoothed distances predicted from the dissimilarities. The final stress value indicates the reliability of the final ordination in relation to the dataset dissimilarities. Final stress values obtained in this study ranged between 10 and 20, which indicate an acceptable ordination with no real risk of misinterpretation for most ecological community datasets. A multi-response permutation procedure (MRPP) was performed using the Sorensen distance measure to test for differences between groups. The chance-corrected within-group agreement index (A) is a proportion between heterogeneity within groups in relation to heterogeneity expected by chance. Thus, indicating accuracy of the clustering within samples of the same group. Values close to zero indicate herogeneity within the group equal expectation by chance, whereas values close to 1 indicate all samples within the groups are identical.

Sequence analysis of partial 16S rRNA genes of endophytic bacteria derived from culture dependent methods and DGGE bands was performed with MEGA version 6.0 (Tamura et al., 2013). Sequences were subsequently compared with those from the GenBank database using BLAST algorithm (Altchul et al., 1997) and used for bacterial identification. The CFU, PLFA abundance, richness and diversity indexes were subjected to analysis of variance (ANOVA) and Tukey's post hoc test using SAS software version 9.6 (Copyright © 2002-2010 SAS Institute Inc. Cary, NC, USA.). Pearson correlation was conducted to determine the relationship between soil properties and bacterial genera abundance (SAS software version 9.6).

3.5. Results

3.5.1. Culturable Bacteria Associated with the Rhizosphere and Root Interior of the Crops

Abundance of culturable bacteria associated with canola, wheat, pea and lentil varied throughout the agricultural fields in Saskatchewan (Fig. 3.1). Rhizosphere bacteria abundance ranged from 10^8-10^{10} CFU·g^{-1} fresh root, whereas root endophytes ranged from 10^4-10^7 CFU·g^{-1} fresh root. The crop species and the location from where the samples were collected, as well as the interaction of these factors, had a significant effect ($P<0.001$) on the rhizospheric bacteria abundance (Table 3.3). For endophytic bacteria abundance, it was not detected differences between locations, but crop species had a significant effect, as well as the interaction of both factors. For instance, the highest bacterial population was obtained in pea ranging from 10^6-10^7 CFU·g^{-1} fresh root in the root interior and 10^9-10^{10} CFU·g^{-1} fresh root in the rhizosphere (Fig. 3.1).

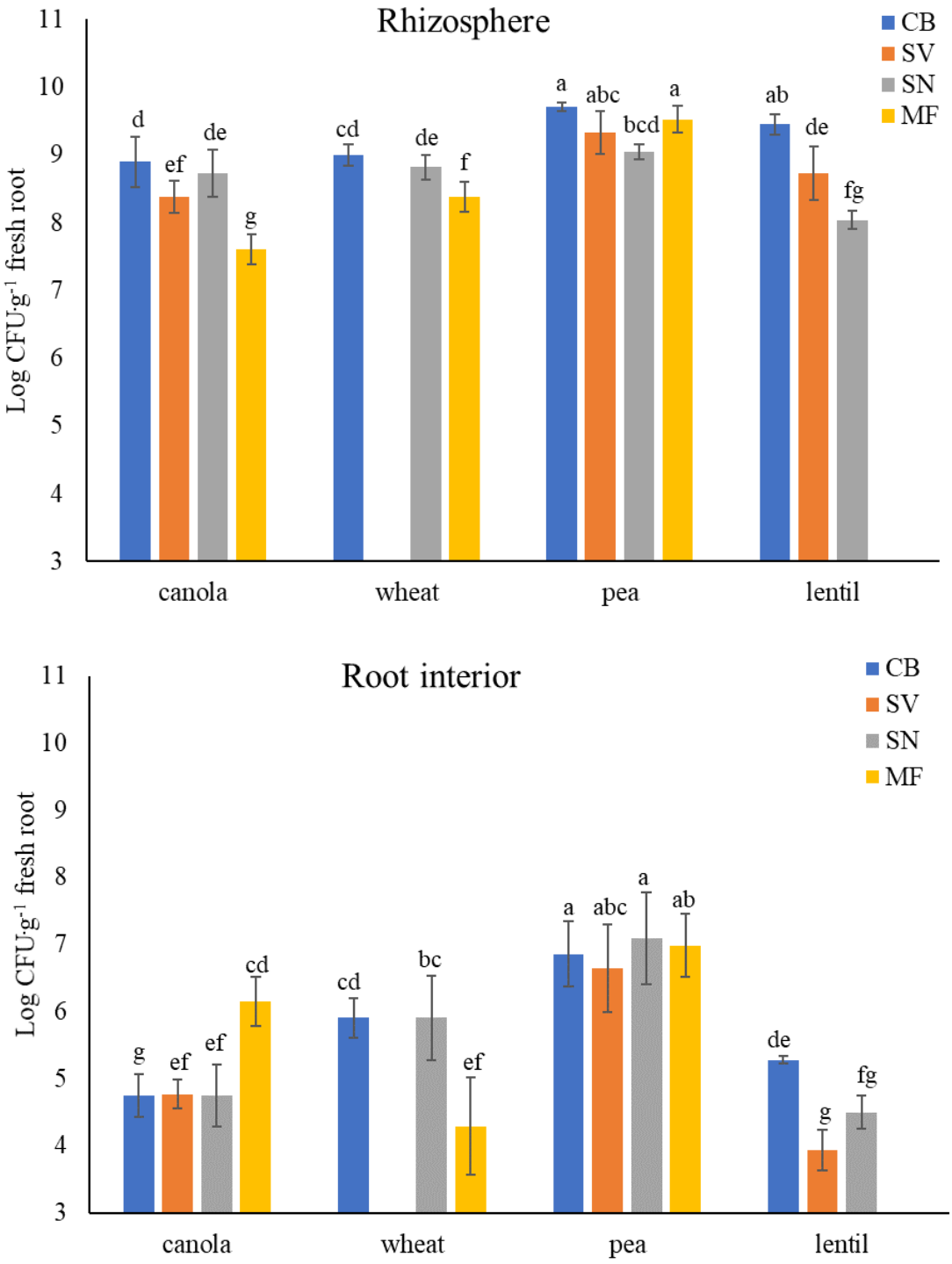

Fig. 3.1. Numbers (Log CFU·g^{-1} fresh root) of rhizosphere and endophytic bacteria associated with crops grown in Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan. Wheat and lentil samples were not collected in SV and MF, respectively. Different letters indicate significant differences at α=0.05 using Tukey's post hoc test.

Table 3.3. ANOVA of the numbers (Log CFU·g^{-1} fresh root) of rhizosphere and endophytic bacteria associated with crops grown in Saskatchewan agricultural fields.

Source of variation	Rhizosphere	Root interior
Location	<0.0001 ***	0.1 n.s.
Crop	<0.0001 ***	<0.0001 ***
Location × Crop	<0.0001 ***	<0.0001 ***

Note: *, **, ***, significant at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., not significant.

A total of 298 endophytic bacteria were identified using 16S rRNA Sanger sequencing (Table B.1, Appendix B). These isolates were classified into 42 genera (Table B.2, Appendix B) using the GenBank Database, from which 26 isolates (42%) were also identified using 16S rRNA high-throughput sequencing. Classification of these isolates detected the phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. Proteobacteria comprised the most prevalent phylum, which included 23 genera and representing 49% of the total number of isolates. Proteobacteria were also present in the four crops growing at all surveyed locations. Within this phylum, the most abundant genera were *Stenotrophomonas* (13%), *Pseudomonas* (7%), and *Pantoea* (7%), followed by *Rhizobium* spp (7%). The phylum Firmicutes corresponded to 26% of the total, being *Bacillus* (14%) and *Paenibacillus* (8%) the most abundant genera. The phylum Actinobacteria represented 24% of the total bacteria strains, being *Microbacterium* (9%) the dominant genus in this group. The genus *Crysobacterium* (<1%), isolated from canola at Melfort, was the only member of the phylum Bacteroidetes (Table B.2, Appendix B).

The number of genera of culturable endophytic bacteria varied among crops (Table B.2, Appendix B). Results indicated the presence of 17 genera in canola, 22 genera in wheat, 17 genera in pea and 9 genera in lentil. *Pseudomonas* and *Microbacterium* were present in all four crops irrespective of the sampling locations. In addition, some genera were more prevalent in certain plant species. For example, *Stenotrophomonas*, *Streptomyces*, *Microbacterium*, *Pseudomonas*, *Bacillus* and *Acinetobacter* were the most common genera found in canola, whereas *Xanthomonas*, *Mycobacterium*, *Brevibacillus* and *Erwinia* were the most prevalent genera in wheat. Similarly, *Pantoea* and *Pseudomonas* were predominant in lentil, whereas *Paenibacillus* was abundant in pea. *Bacillus*, *Rhizobium* and *Microbacterium* had a higher abundance in both lentil and pea. Results also indicated that crops growing on different locations supported distinct bacterial genera

(Table B.2, Appendix B). For example, crops from Central Butte and Stewart Valley contained 21 and 13 genera, respectively, whereas crops from Saskatoon and Melfort contained 17 genera each. The most abundant genera also varied among locations. For example, *Stenotrophomonas* was found to be the dominant genus in Central Butte and Melfort, but *Microbacterium* and *Pantoea* were also abundant in Central Butte. Additionally, *Bacillus* and *Paenibacillus* were highly abundant in Stewart Valley and Saskatoon, and *Streptomyces* was dominant at the Saskatoon site.

3.5.2. PLFA Profiles of Soil Bacterial Communities

Total, bacterial, G+ and G- PLFA biomass (nmol·g^{-1} soil) differed ($P<0.01$) between locations but not between crops (Table 3.4). Soil collected from Melfort exhibited greater total, bacterial, G+ and G- PLFA biomass as compared to remaining three locations, irrespectively of the crop type (Tables 3.4). Relative abundance (mol %) of bacteria PLFA did not vary among samples (Tables 3.4), however, G+ and G- bacteria PLFA biomarkers related to specific crop species differed ($P<0.01$) depending on the locations (Table 3.5). For example, pea plants had the greatest abundance of G+ bacteria in Central Butte, but also had the lowest abundance in Stewart Valley. Similarly, wheat plants had the greatest abundance of G- PLFA in Central Butte but had the lowest values in Melfort (Fig. 3.2). Fungal PLFA biomass and relative abundance related to specific crops species differed ($P<0.01$) depending on the locations (Table 3.6). The relative abundance of AMF PLFA biomass did not vary among samples; however, the AMF PLFA biomass related to specific crops species differed ($P<0.01$) depending on the crop species (Table 3.7).

Table 3.4. Total and bacterial PLFA determined in bulk soils associated with canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

Location	Crop	Total PLFA ($nmol \cdot g^{-1}$ soil)		Bacterial PLFA ($nmol \cdot g^{-1}$ soil)		Bacterial PLFA (mol %)		G+ PLFA ($nmol \cdot g^{-1}$ soil)		G- PLFA ($nmol \cdot g^{-1}$ soil)	
Central Butte	Canola	19.7 ± 3.4	n.s.	9.5 ± 0.8	n.s.	49.1 ± 5.9	n.s.	3.1 ± 0.8	n.s.	7.0 ± 1.1	n.s.
	Wheat	19.8 ± 10.8	n.s.	9.3 ± 4.4	n.s.	48.0 ± 5.4	n.s.	2.9 ± 1.6	n.s.	7.6 ± 4.0	n.s.
	Pea	6.5 ± 3.9	n.s.	3.4 ± 2.4	n.s.	54.5 ± 3.2	n.s.	1.2 ± 0.9	n.s.	2.0 ± 1.1	n.s.
	Lentil	13.8 ± 6.9	n.s.	7.1 ± 2.6	n.s.	53.5 ± 5.7	n.s.	2.7 ± 1.2	n.s.	4.4 ± 2.2	n.s.
	Average	15.0 ± 6.2	b	7.4 ± 2.5	c	51.3 ± 5.1	n.s.	2.5 ± 1.1	c	5.2 ± 2.1	b
Stewart Valley	Canola	25.4 ± 4.6	n.s.	12.3 ± 2.1	n.s.	48.4 ± 2.6	n.s.	4.1 ± 0.9	n.s.	8.7 ± 2.0	n.s.
	Pea	15.8 ± 1.9	n.s.	9.1 ± 1.4	n.s.	57.4 ± 2.6	n.s.	2.3 ± 0.6	n.s.	5.8 ± 0.6	n.s.
	Lentil	24.2 ± 5.6	n.s.	12.6 ± 2.1	n.s.	52.8 ± 4.5	n.s.	3.9 ± 1.0	n.s.	8.4 ± 2.0	n.s.
	Average	21.8 ± 4.0	b	11.3 ± 1.9	bc	52.9 ± 3.2	n.s.	3.5 ± 0.8	bc	7.6 ± 1.5	ab
Saskatoon	Canola	38.8 ± 16.1	n.s.	15.4 ± 7.7	n.s.	49.3 ± 5.7	n.s.	5.6 ± 2.9	n.s.	11.9 ± 6.7	n.s.
	Wheat	23.0 ± 8.0	n.s.	12.0 ± 3.8	n.s.	53.3 ± 5.9	n.s.	4.4 ± 1.4	n.s.	7.6 ± 2.9	n.s.
	Pea	22.4 ± 9.9	n.s.	10.7 ± 3.9	n.s.	49.2 ± 6.3	n.s.	3.9 ± 1.7	n.s.	7.6 ± 3.2	n.s.
	Lentil	17.1 ± 7.9	n.s.	9.2 ± 3.7	n.s.	54.7 ± 3.0	n.s.	3.1 ± 1.4	n.s.	5.7 ± 2.4	n.s.
	Average	25.3 ± 10.5	b	11.8 ± 4.8	b	51.6 ± 5.2	n.s.	4.2 ± 1.8	b	8.2 ± 3.8	ab
Melfort	Canola	29.6 ± 5.1	n.s.	15.7 ± 2.8	n.s.	53.0 ± 0.5	n.s.	5.8 ± 1.3	n.s.	8.7 ± 1.3	n.s.
	Wheat	37.8 ± 11.6	n.s.	19.7 ± 6.5	n.s.	51.8 ± 1.9	n.s.	7.6 ± 2.9	n.s.	10.6 ± 3.4	n.s.
	Pea	31.3 ± 14.3	n.s.	17.1 ± 6.2	n.s.	52.5 ± 1.7	n.s.	6.3 ± 2.6	n.s.	9.7 ± 3.2	n.s.
	Average	32.9 ± 10.3	a	17.5 ± 5.2	a	49.7 ± 1.4	n.s.	6.6 ± 2.2	a	9.7 ± 2.6	a
	ANOVA										
	Location	0.001 ***		0.004 **		0.7 n.s.		<0.0001 ***		0.004 ***	
	Crop	0.4 n.s.		0.3 n.s.		0.3 n.s.		0.3 n.s.		0.2 n.s.	
	Location × Crop	0.7 n.s.		0.7 n.s.		0.1 n.s.		0.5 n.s.		0.7 n.s.	

Note: *, **, ***, significant at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., not significant. Different letters indicate significant differences at $P \leq 0.05$, using Tukey's post hoc test.

Table 3.5. ANOVA of the relative abundance of bacterial G+ and G- PLFA (mol%) determined in Saskatchewan agricultural soils.

Source of variation	G+	G-
Location	0.0002 ***	0.005 **
Crop	0.8 n.s.	0.3 n.s.
Location × Crop	0.01 *	0.01 **

Note: *, **, ***, significant at $P \leq 0.05, 0.01, 0.001$, respectively. n.s, not significant.

Analysis of PLFA profiles using NMDS ordination resulted in a 2-dimensional solution with a final stress of 12.7 (Fig. 3.3). Ordination along Axis 1 and to some extent along Axis 2 has allowed the separation of soil bacterial communities based on the sampling locations. Bacterial, G+ and G- PLFA biomass, as well as the soil organic matter and silt contents were positively correlated with Melfort profiles, whereas, pH and sand content were positively correlated with Central Butte (Fig. 3.3).

3.5.3. Community Structure of Endophytic, Rhizosphere and Bulk Soil Bacteria Associated with Crops using DGGE

The NMDS analysis of DGGE profiles derived from endophytic, rhizosphere and bulk soil bacterial communities resulted in a 3-dimensional solution and final stress of 20.5 (Fig. 3.4). Two-dimensional ordination along Axis 1 and 2 revealed that root endophytes, rhizosphere and bulk soil bacteria were clustered separately; however, the rhizosphere bacteria communities were greatly overlapped by bulk soil bacteria. For all crop species, the endophytic DGGE band patterns consisted of several intense and/or lower number of DGGE bands (Fig 3.5), indicating that the 16S rRNA fragments of only few bacterial populations were dominant in the root interior. In contrast, the bulk soil and rhizosphere band patterns consisted of few intense bands and many less intense bands (Figs. 3.6 and 3.7), indicating that in rhizosphere and bulk soil samples mainly consisted of bacterial populations which were less prevalent and equally abundant. Due to these differences, the DGGE profiles of endophytic, bulk soil and rhizosphere bacteria were analyzed separately.

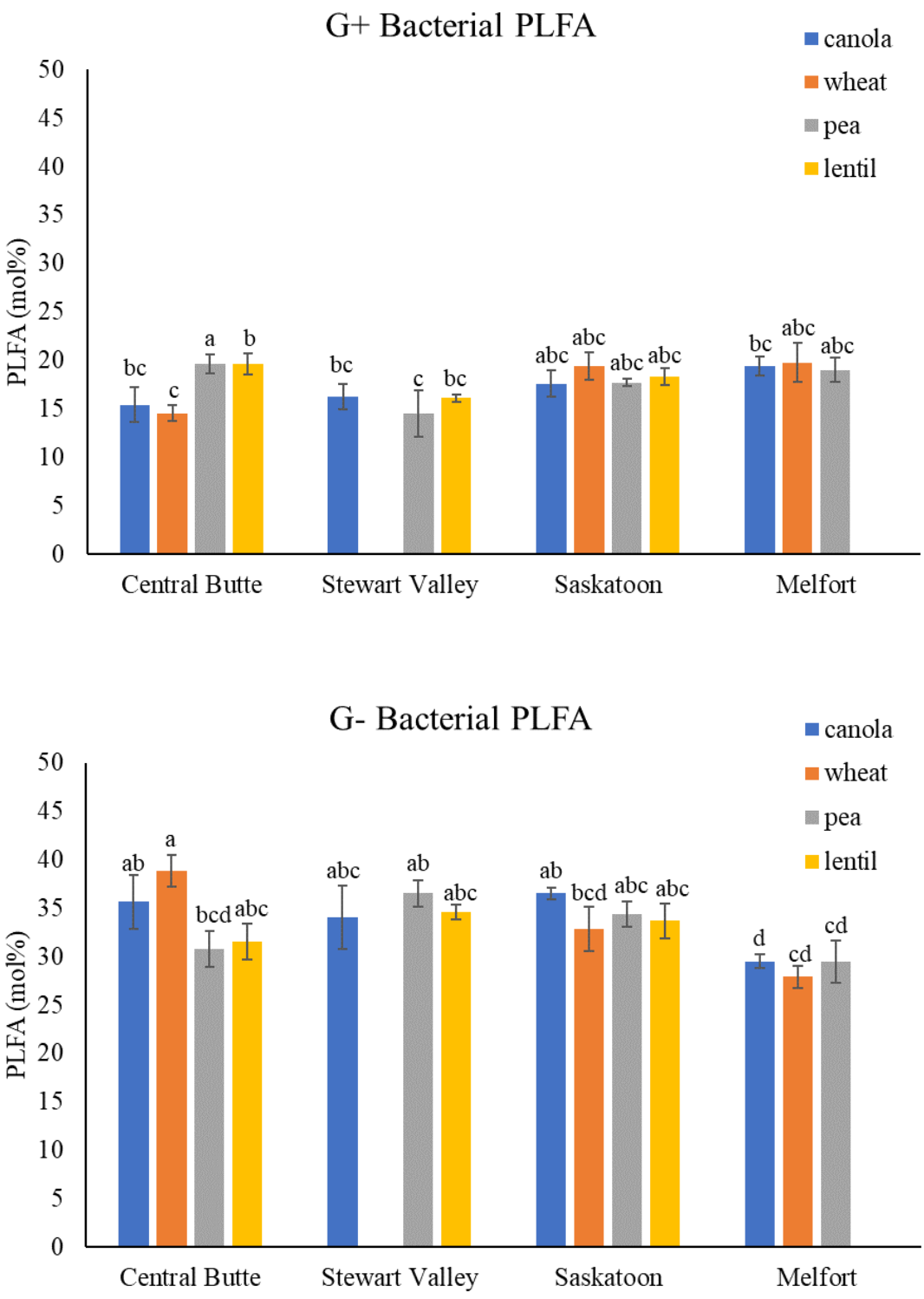

Fig. 3.2. Relative abundance (mol%) of G+ and G- bacterial in soils collected in Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan, cultivated with wheat, canola, pea and lentil. Wheat and lentil samples were not collected in SV and MF, respectively. Different letters indicate significant differences among samples at α=0.05 using Tukey's post hoc test.

Table 3.6. Fungal PLFA determined in bulk soils associated with canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

		Fungal PLFA							
Location	Crop	($nmol \cdot g^{-1}$ soil)				(mol %)			
Central Butte	Canola	0.75	±	0.07	n.s.	3.10	±	1.35	b
	Wheat	0.83	±	0.51	n.s.	5.89	±	1.07	a
	Pea	0.16	±	0.15	n.s.	1.49	±	0.83	bcd
	Lentil	0.49	±	0.17	n.s.	2.46	±	0.46	b
	Average	0.56	±	0.22	b	3.23	±	0.93	a
Stewart Valley	Canola	1.06	±	0.18	n.s.	2.20	±	0.56	bcd
	Pea	0.66	±	0.14	n.s.	1.79	±	0.18	d
	Lentil	0.90	±	0.19	n.s.	2.29	±	0.91	bcd
	Average	0.87	±	0.17	ab	2.09	±	0.55	b
Saskatoon	Canola	1.13	±	0.90	n.s.	2.02	±	0.24	bcd
	Wheat	0.65	±	0.38	n.s.	2.65	±	0.84	bcd
	Pea	0.68	±	0.31	n.s.	2.58	±	0.48	bcd
	Lentil	0.70	±	0.30	n.s.	3.31	±	1.13	b
	Average	0.79	±	0.47	ab	2.64	±	0.67	ab
Melfort	Canola	1.13	±	0.23	n.s.	1.90	±	0.44	d
	Wheat	1.36	±	1.18	n.s.	2.86	±	0.89	bcd
	Pea	1.05	±	0.49	n.s.	2.47	±	0.92	bcd
	Average	1.18	±	0.63	a	2.41	±	0.75	ab
	ANOVA								
	Location	0.005 **				0.001 ***			
	Crop	0.3 n.s.				0.003 ***			
	Location × Crop	0.5 n.s.				0.01 **			

Note: *, **, ***, significant at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., not significant. Different letters indicate significant differences at $P \leq 0.05$, using Tukey's post hoc test.

Table 3.7. AMF PLFA determined in bulk soils associated with canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

Location	Crop	AMF PLFA (nmol·g^{-1} soil)				AMF PLFA (mol %)			
Central Butte	Canola	0.68	±	0.44	ab	0.70	±	0.03	n.s.
	Wheat	1.19	±	0.66	a	0.72	±	0.02	n.s.
	Pea	0.09	±	0.15	b	0.45	±	0.39	n.s.
	Lentil	0.28	±	0.05	b	0.74	±	0.08	n.s.
	Average	0.56	±	0.33	n.s.	0.65	±	0.13	n.s.
Stewart Valley	Canola	0.52	±	0.14	ab	0.69	±	0.01	n.s.
	Pea	0.28	±	0.04	b	0.71	±	0.04	n.s.
	Lentil	0.56	±	0.31	ab	0.69	±	0.03	n.s.
	Average	0.46	±	0.16	n.s.	0.70	±	0.03	n.s.
Saskatoon	Canola	0.57	±	0.36	ab	0.68	±	0.03	n.s.
	Wheat	0.63	±	0.36	ab	0.58	±	0.07	n.s.
	Pea	0.48	±	0.17	ab	0.67	±	0.03	n.s.
	Lentil	0.43	±	0.12	ab	0.73	±	0.05	n.s.
	Average	0.53	±	0.25	n.s.	0.66	±	0.05	n.s.
Melfort	Canola	0.54	±	0.14	ab	0.70	±	0.01	n.s.
	Wheat	1.17	±	0.64	a	0.62	±	0.25	n.s.
	Pea	0.81	±	0.32	ab	0.56	±	0.15	n.s.
	Average	0.81	±	0.37	n.s.	0.63	±	0.14	n.s.
	ANOVA								
	Location	0.1 n.s.				0.4 n.s.			
	Crop	0.01 **				0.4 n.s.			
	Location × Crop	0.03 *				0.3 n.s.			

Note: *, **, ***, significant at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., not significant. Different letters indicate significant differences at $P \leq 0.05$, using Tukey's post hoc test.

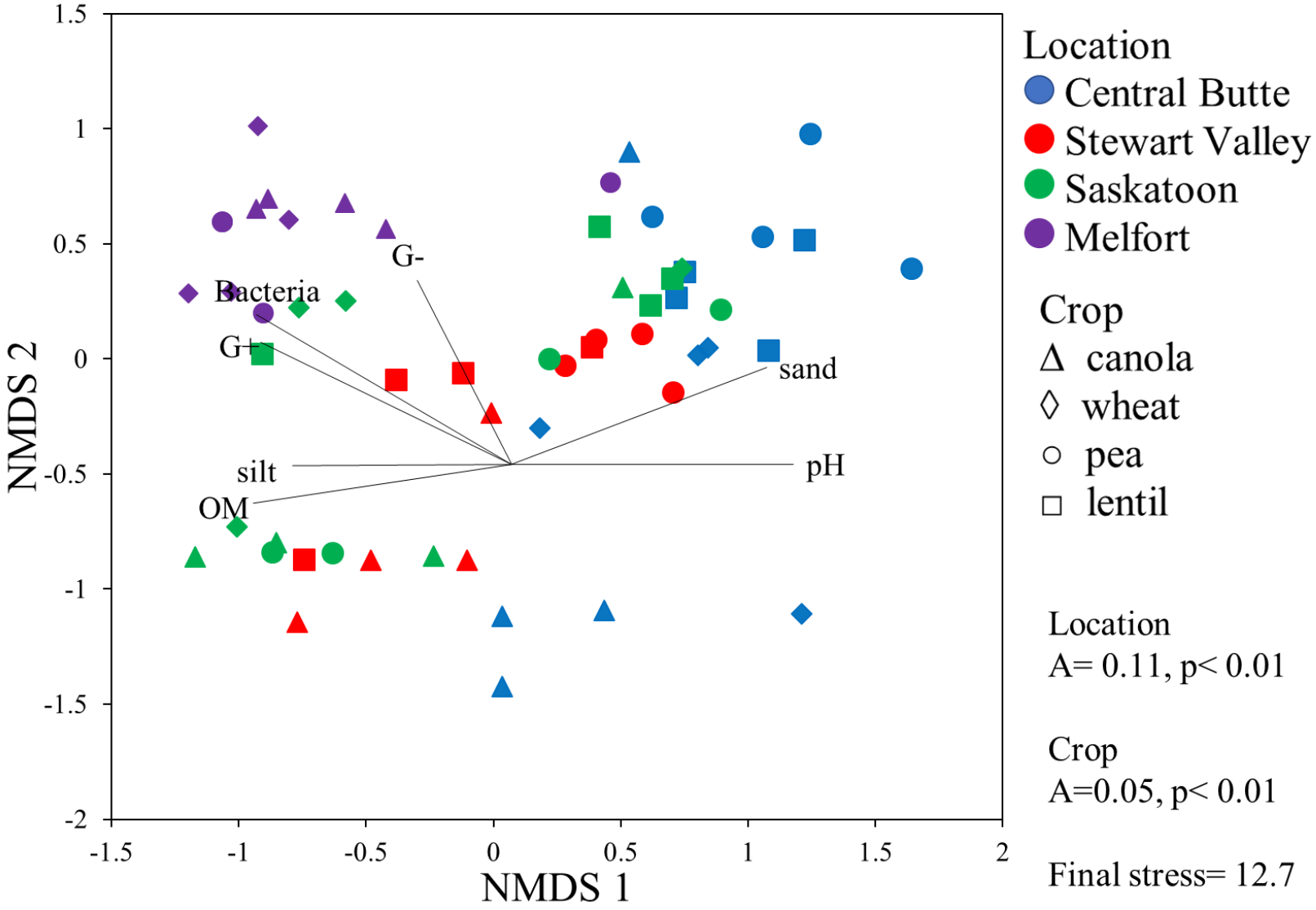

Fig. 3.3. Non-metric multidimensional scaling (NMDS) ordination analysis and multiple response permutation procedure (MRPP) of PLFA profiles (mol%) in soils collected in Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

Community structure of endophytic bacteria assessed using DGGE (based on the band fingerprint) indicated distinct population profiles amongst crops and locations. The Dendrogram analysis of DGGE band pattern (Fig. 3.5) indicated that samples from the same crop species clustered together. For example, community profiles from wheat and canola clustered at approximately 10% and 60 % of similarity, respectively, whereas pea and lentil profiles produced individual clusters at 90% of similarity. The NMDS analysis resulted in a 3-dimensional solution and final stress of 17.9 (Fig. 3.8). The MRPP test was used to establish a priori differences between crops or locations. Analysis of ordination using crop types as a grouping variable indicated a higher A value (A=0.15**) compared to locations (A=0.02**), indicating that heterogeneity within groups is lower when samples are clustered by crops types. Two-dimensional ordination along Axis 2 and 3 revealed that pea and lentil formed individual groups, whereas wheat and canola profiles overlapped. Conversely, endophytic communities associated with wheat grown at Central Butte were separated on an individual cluster.

Dominant endophytic populations (n=113), identified by 16S rRNA Sanger sequencing of prominent DGGE bands (Table B.3, Appendix B), revealed that a total of 10 dominant genera corresponded to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes (Fig. 3.9). The phylum Proteobacteria was the most dominant phylum in all endophytic samples, whereas the phylum Bacteroidetes, represented by *Flavobacterium* sp., was only associated with wheat plants. The dominant genera assessed by DGGE included *Pseudomonas*, a predominant genus in canola, *Rhizobium* predominant in pea and lentil, whereas *Pseudomonas* and *Arthrobacter* were mostly abundant in wheat. Additionally, DGGE bands that corresponded to unculturable bacteria also were detected in all studied crops (Fig. 3.9).

Similarly to endophytes, rhizosphere bacterial communities also exhibited distinct DGGE profiles amongst crops and locations. The dendrogram analysis of DGGE band pattern of rhizosphere communities (Fig. 3.6) indicated that samples from wheat and lentil clustered at approximately 90% and 10 % of similarity, respectively, whereas in pea and canola the clusters were overlaping, producing a single cluster at 30% of similarity. The NMDS analysis of rhizosphere communities resulted in a 3-dimensional solution and final stress of 12.40 (Fig. 3.10). Analysis of ordination using crop types as a grouping variable indicated a higher A value (A=0.11**) compared to locations (A=0.04**), indicating lower heterogeneity (Fig. 3.10). Two-dimensional ordination along Axis 1 and 3 revealed an overlapping of wheat and canola profiles,

whereas pea and lentil resulted in individual groups. Additionally, rhizosphere communities associated with wheat grown at Central Butte were separated on an individual cluster.

Similar to endophytic and rhizosphere bacterial profiles, dendrogram analysis of bulk soil microbial fingerprints also indicated distinct profiles among crops species and locations (Fig. 3.7), consisting of two clusters; one represented by pea at approximately 90% and another representing the remaining crops at 40% of similarity. The NMDS analysis of rhizosphere communities resulted in a 3-dimensional solution and final stress of 15.1 (Fig. 3.11). Analysis of ordination using crop types and locations as a grouping variable indicated a higher A value for crops compared to locations (0.20** *vs*. 0.03**, respectively), indicating lower heterogeneity for the crops studied. In fact, two-dimensional ordination along Axis 2 and 3 demonstrated that lentil and pea formed individual groups, but wheat and canola profiles overlapped each other.

3.5.4. Community Structure and Diversity of Bacterial Communities Associated with Crops assessed by 16S rRNA High-Throughput Sequencing

Processing of 16S rRNA high-throughput Illumina sequencing generated 5,261,433 high quality sequence reads. A total of 12,549 OTUs were detected, which corresponded to 11,932 OTUs and 3,491 OTUs in 96 rhizosphere and/or root samples, respectively (data not shown). Venn diagram representing OTU distribution in the rhizosphere and root interior of each crop, revealed that canola harbored the highest number of OTUs, followed by wheat, pea and lentil (Fig. 3.12). The number of OTUs of rhizosphere bacteria in all crops was higher in relation to root endophytes. Additionally, some OTUs were shared between the rhizosphere and root interior. However, these OTUs represented a high percentage (97-99%) and (77-90%) of the sequence reads in the root interior and rhizosphere, respectively. Venn diagram also revealed that number of OTUs that were detected only in the rhizosphere of wheat and canola was higher when compared to pea and lentil separately (Fig. 3.12). Similarly, the proportion of OTUs detected in the root interior of canola and wheat was higher when compared to pea and lentil.

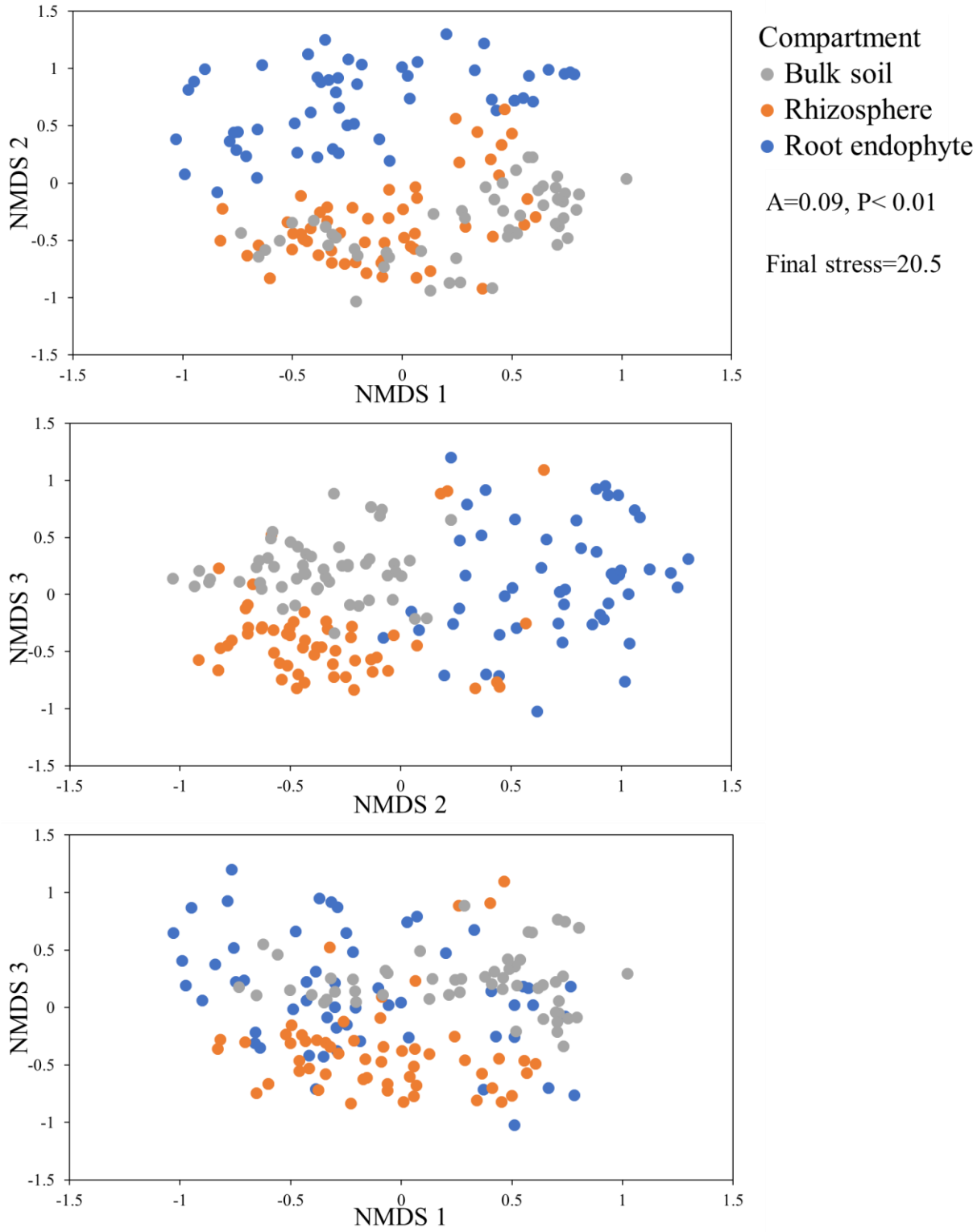

Fig. 3.4. Non-metric multidimensional scaling (NMDS) analysis for DGGE band patterns of endophytic, rhizosphere and bulk soil bacteria 16S rRNA communities in canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

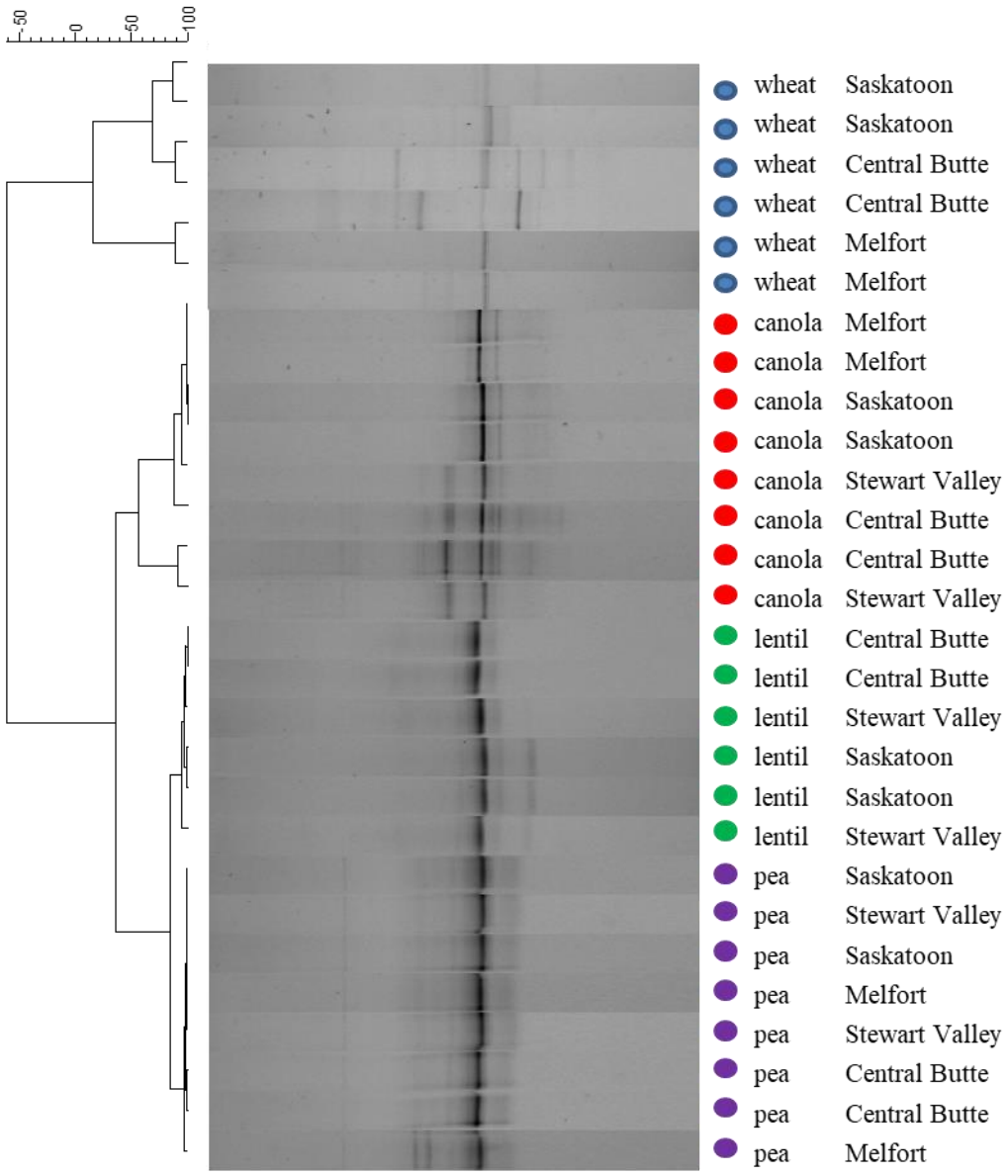

Fig. 3.5. Dendrogram analysis using Pearson's correlation for DGGE banding patterns of endophytic bacterial 16S rRNA communities in canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

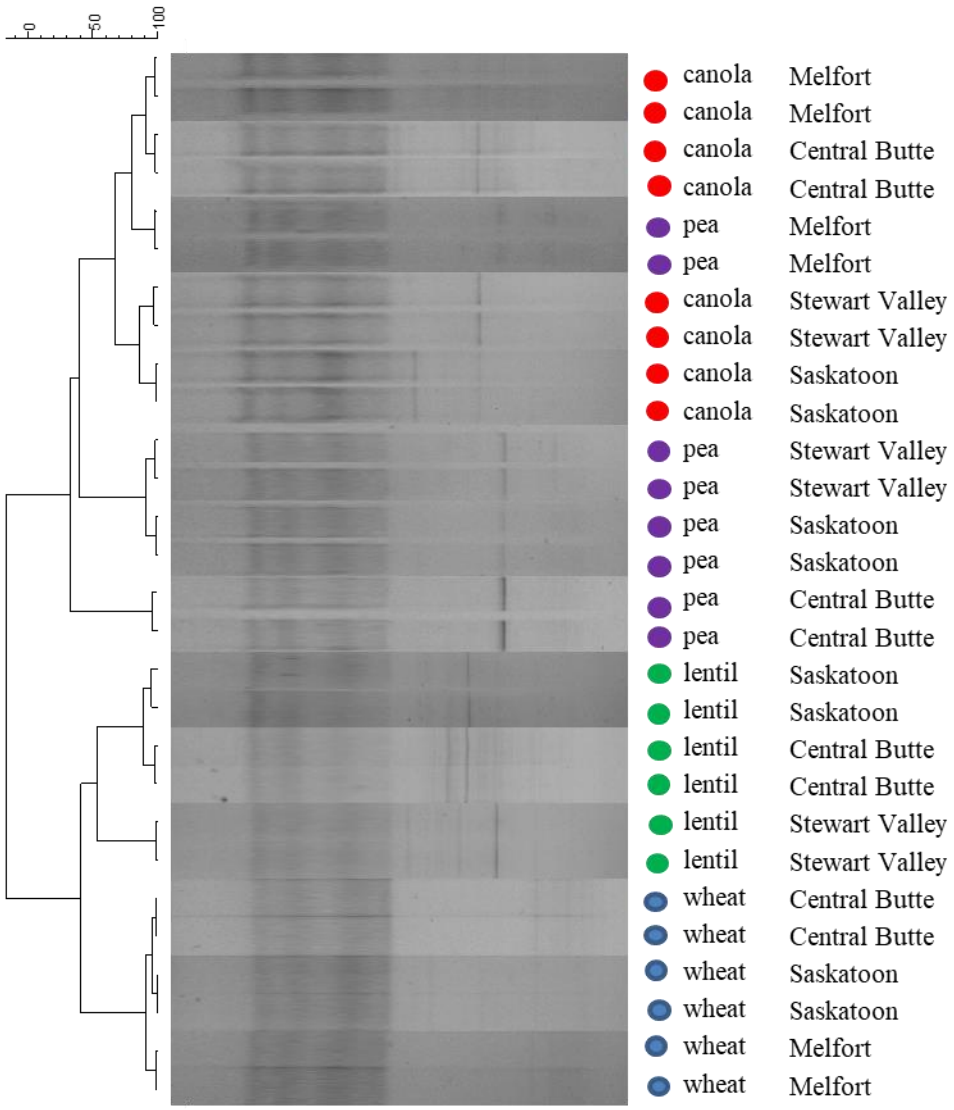

Fig. 3.6. Dendrogram analysis using Pearson's correlation for DGGE banding patterns of rhizosphere bacterial 16S rRNA communities in canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

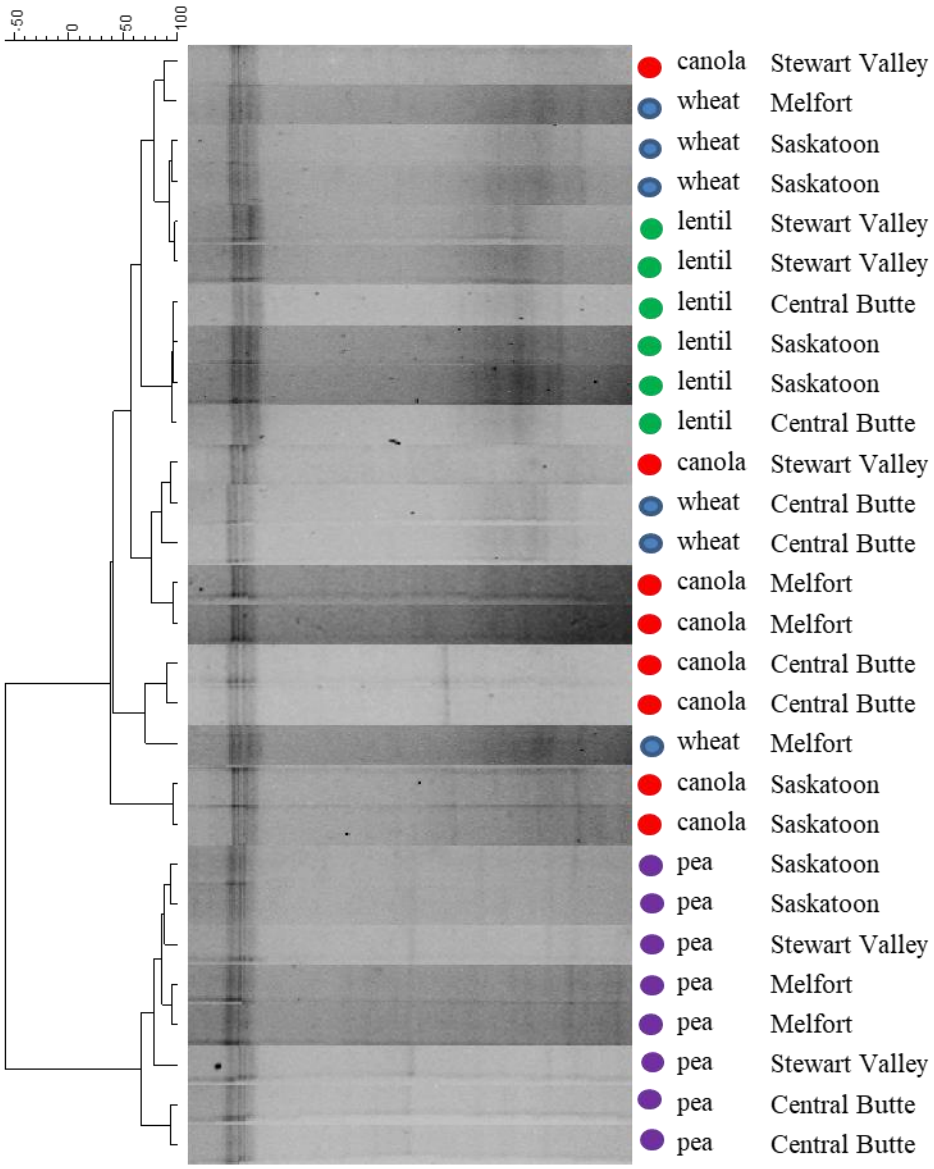

Fig. 3.7. Dendrogram analysis using Pearson's correlation for DGGE banding patterns of bulk soil bacterial 16S rRNA communities in canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

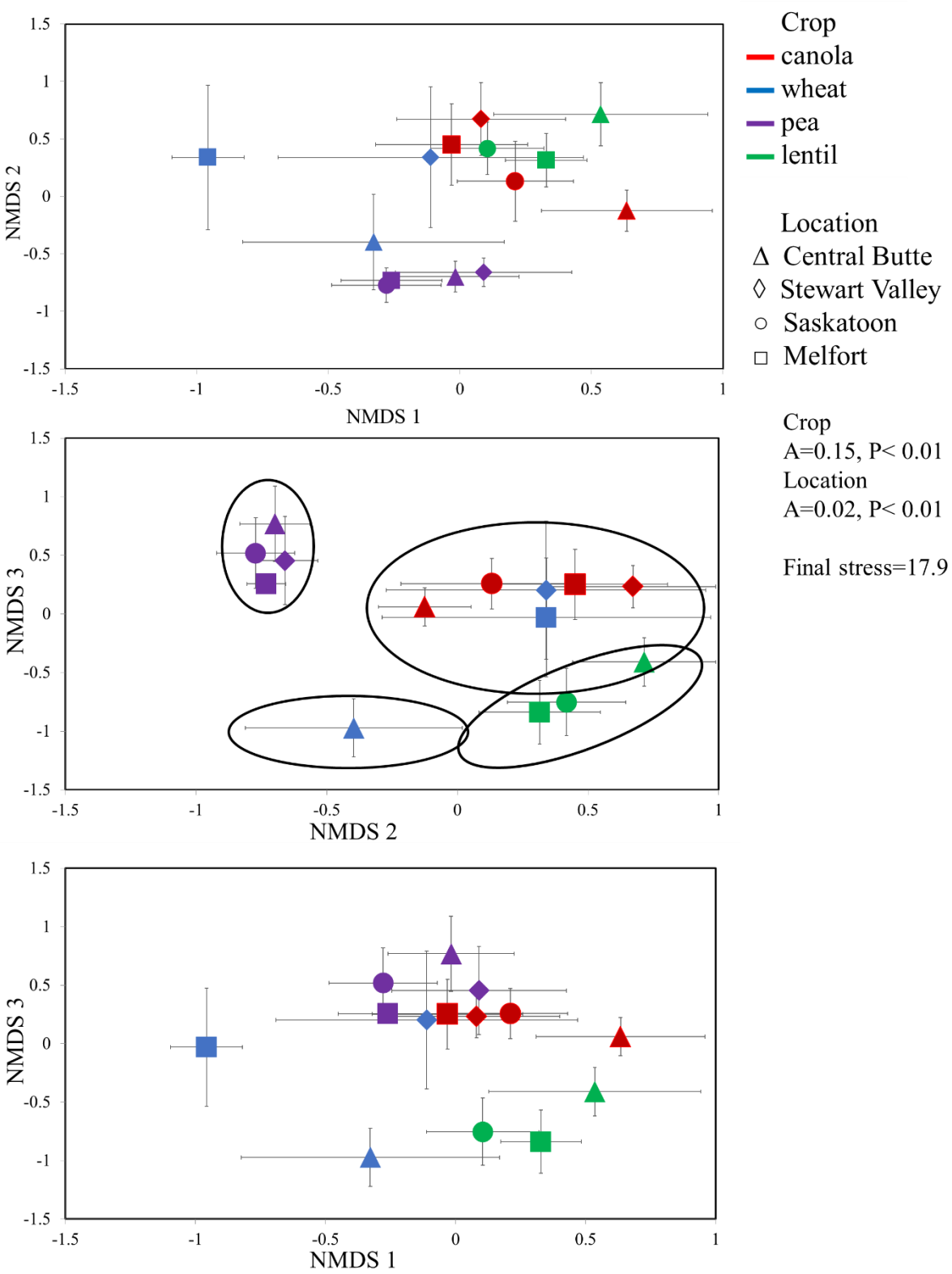

Fig. 3.8. Non-metric multidimensional scaling (NMDS) analysis for DGGE banding patterns of endophytic bacterial 16S rRNA communities in canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

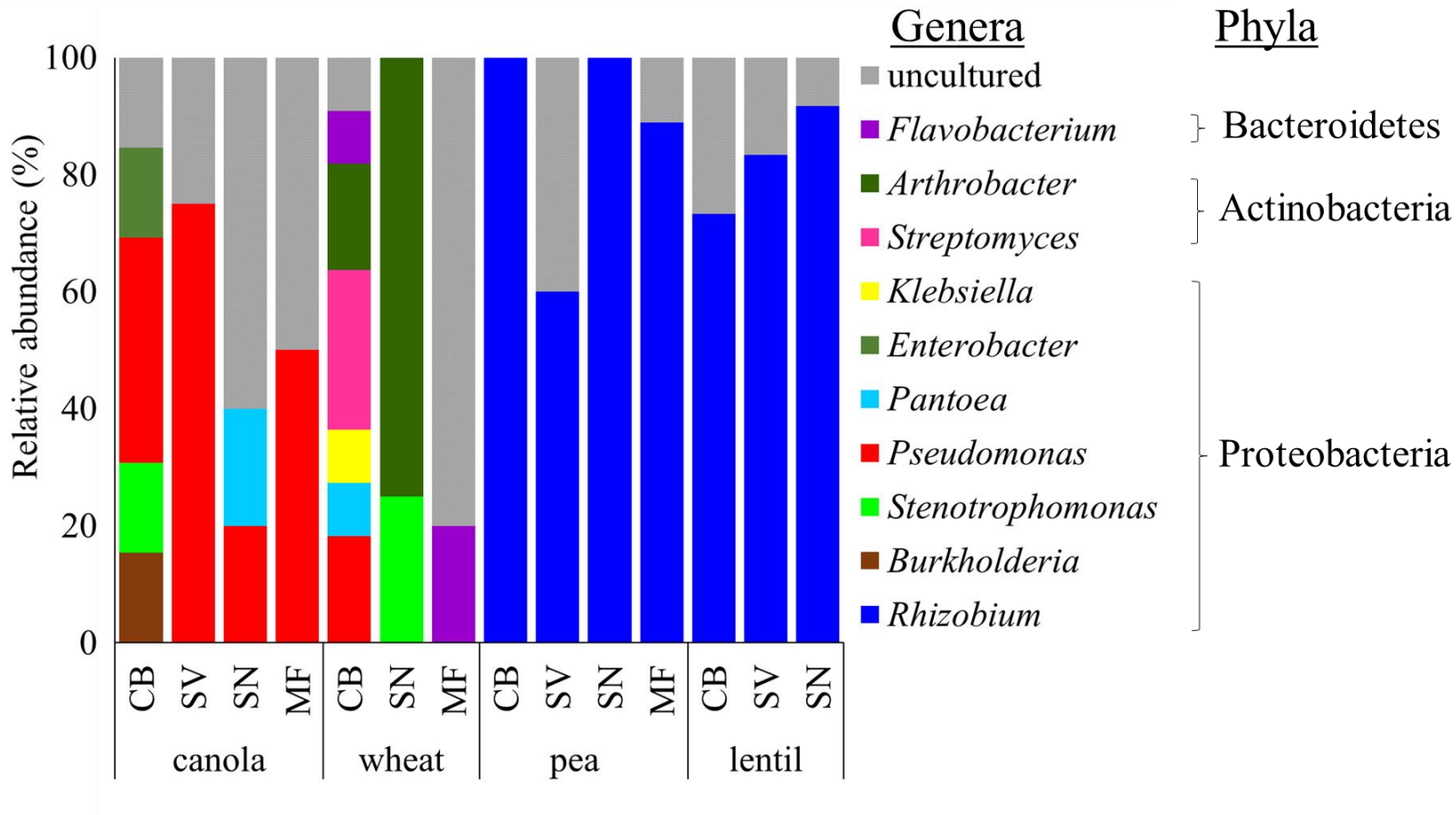

Fig. 3.9. Relative abundance of dominant bacterial endophyte genera associated with wheat (n=21), canola (n=29), lentil (n=40) and pea (n=23) collected at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan. Dominant genera were determined using DGGE.

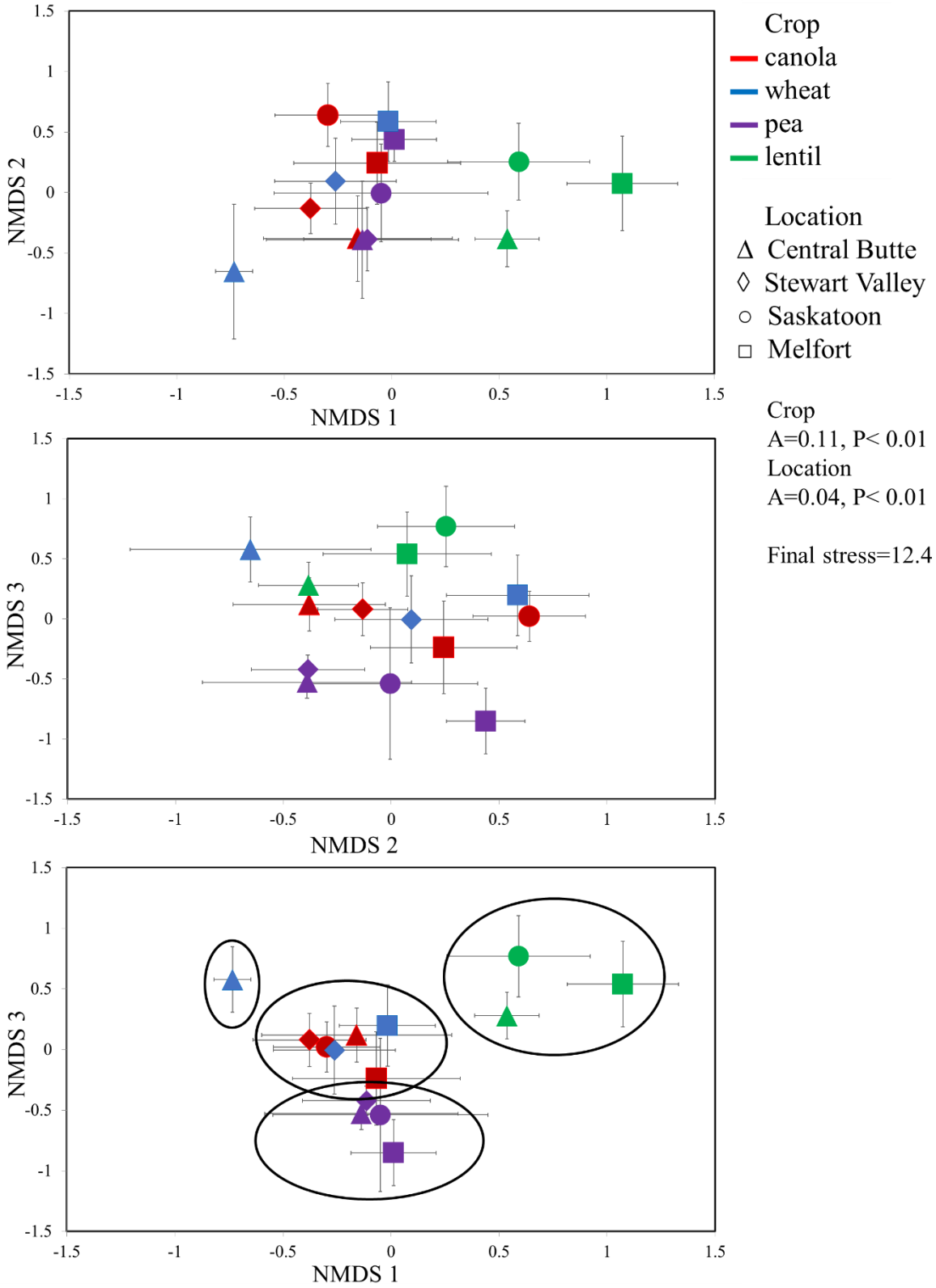

Fig. 3.10. Non-metric multidimensional scaling (NMDS) analysis for DGGE banding patterns of rhizosphere bacterial 16S rRNA communities in canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

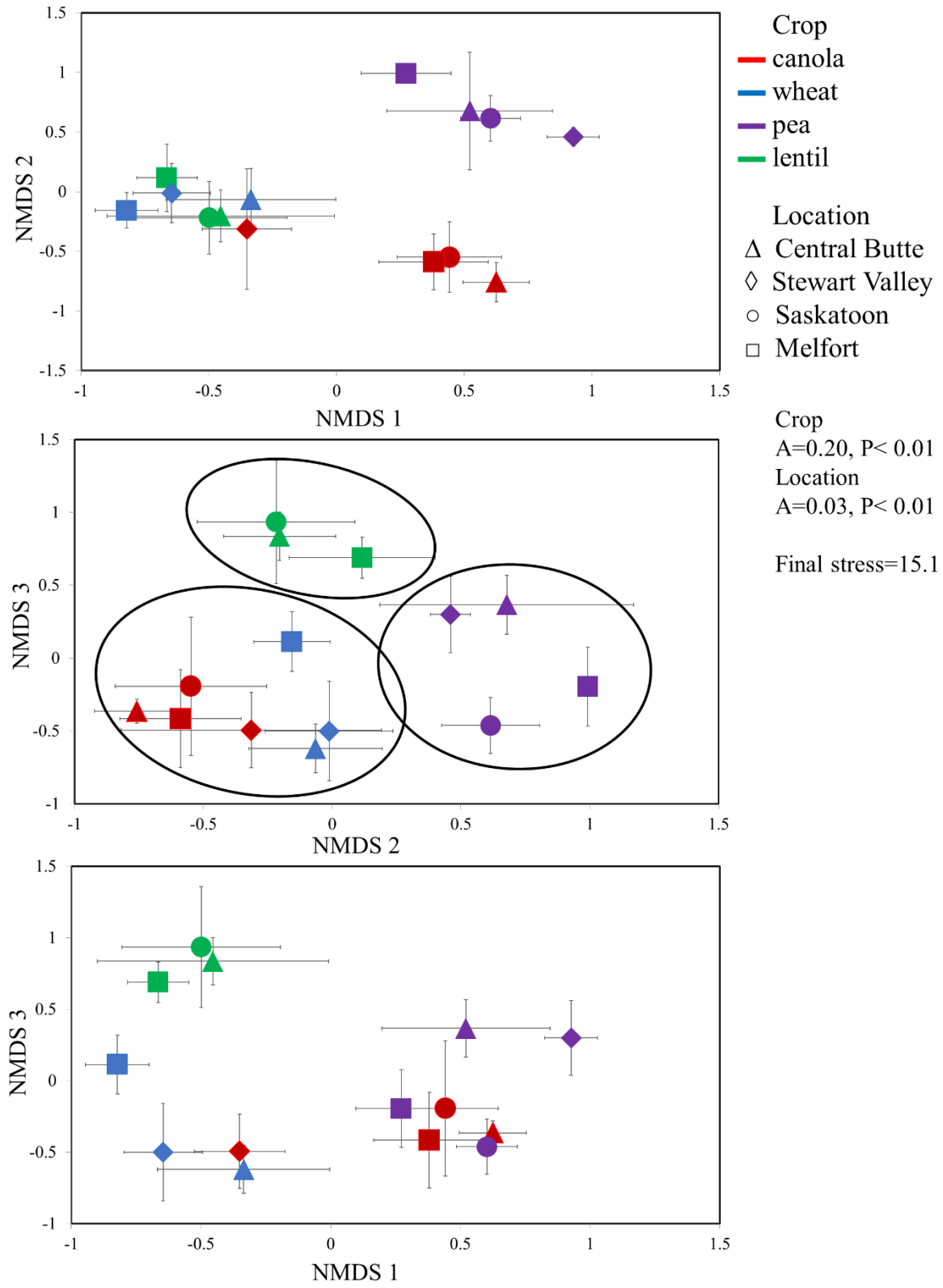

Fig. 3.11. Non-metric multidimensional scaling (NMDS) analysis for DGGE banding patterns of bulk soil bacterial 16S rRNA communities in canola, wheat, pea and lentil collected at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

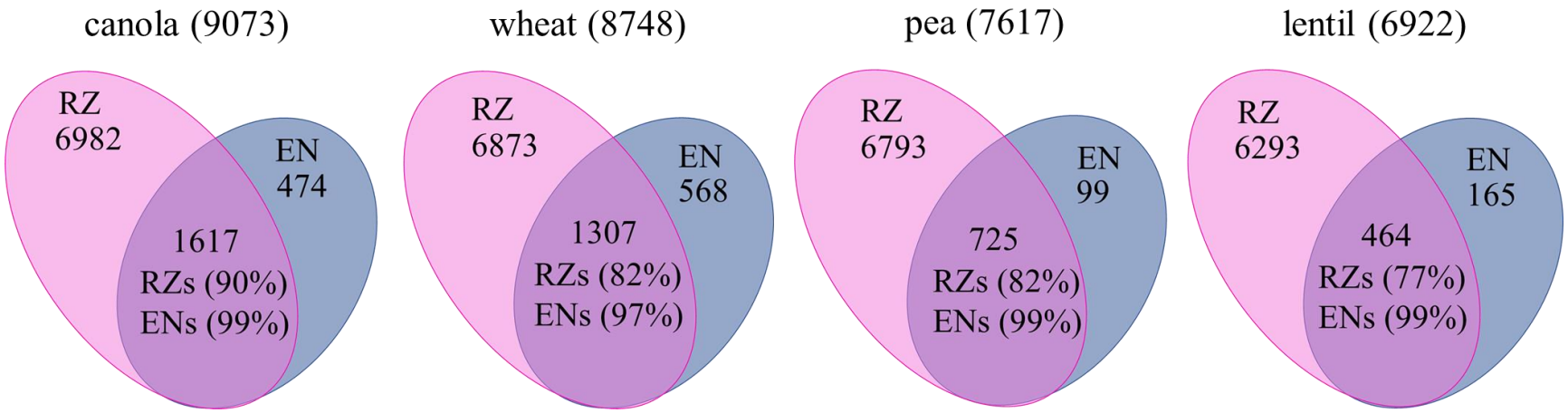

Fig. 3.12. Venn diagram representing bacterial OTUs associated with the rhizosphere (RZ) and root interior (EN) of canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan. Percentage of read counts, included between parenthesis, indicates the proportion of sequence reads associated with the bacterial OTUs that were detected in both rhizo-compartments, in relation to the total number of sequence reads in the rhizosphere (RZs) and/or root interior (ENs).

The diversity of bacterial communities associated with crops was evaluated using Chao 1 estimator and Simpson's reciprocal (1/D) index (Table 3.8). Chao1 estimates the species richness and takes into consideration underrepresented OTUs (singletons and doubletons), whereas Simpson's reciprocal (1/D) index takes into consideration total number of OTUs and their relative abundance. Bacterial communities of the rhizosphere from the crops exhibited significantly higher ($P<0.001$) diversity and species richness compared to the root interior (Table 3.9). Rhizosphere communities were significantly influenced by the interaction of soil and crops (Table 3.9) based on Chao1 ($P=0.0075$) and 1/D index ($P<0.001$). The highest number of OTUs and diversity in the rhizosphere was observed in the wheat collected in Central Butte. However, the lowest number of OTUs was observed in pea collected at Stewart Valley, but the diversity index was the lowest in the same crop collected in Central Butte. Diversity of root bacterial endophytes based on 1/D index was significantly influenced ($P=0.0185$) by the interaction of crop and location whereas Chao1 richness was only significantly different ($P=0.0003$) between crop species (Table 3.9). In the root interior, the diversity and number of OTUs were higher in canola, followed by wheat, pea and lentil (Table 3.8).

The community structure of bacterial OTUs from rhizosphere communities, determined using principal coordinate analysis (PCoA), resulted in a 3-dimensional solution in which PC1, PC2 and PC3 exhibited a variation of 20 %, 13 % and 11 %, respectively (Fig. 3.13). Rhizosphere bacteria exhibited high variability in the OTU profiles among all crops and locations studied. As a result, no clustering was identified in response to these factors. Similarly, the PCoA of root endophytic bacteria also resulted in a 3-dimensional solution (Fig. 3.14), in which PC1, PC2 and PC3 accounted for 52 %, 17 % and 8 %, respectively. Bacterial communities associated with the root interior were clustered in 3 regions, corresponding to canola, wheat, and a cluster containing pea and lentil communities. However, no clustering was detected between endophytic communities from different field locations.

Table 3.8. Richness (Chao 1) and diversity (1/D) of rhizosphere and root endophytic bacteria in canola, wheat, pea and lentil grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

		Rhizosphere				Root endophytes			
Crop	Location	Chao1		1/D		Chao1		1/D	
Canola	CB	2084	ab	97.1	bc	350	n.s.	5.9	n.s.
	SV	2105	ab	72.6	cd	293	n.s.	6.8	n.s.
	SN	1998	ab	33.5	ef	174	n.s.	2.7	n.s.
	MF	1561	b	54.0	de	332	n.s.	4.2	n.s.
	Average	1937	ab	64.3	b	288	a	4.9	a
Wheat	CB	2673	a	140.4	a	413	n.s.	5.3	n.s.
	SN	2201	ab	113.9	ab	277	n.s.	3.4	n.s.
	MF	2098	ab	111.7	ab	371	n.s.	5.0	n.s.
	Average	2324	a	122.0	a	354	a	4.6	a
Pea	CB	1742	b	6.8	f	206	n.s.	1.1	n.s.
	SV	1422	b	9.6	f	135	n.s.	1.0	n.s.
	SN	2236	ab	30.8	ef	118	n.s.	1.0	n.s.
	MF	1669	b	55.5	de	147	n.s.	1.0	n.s.
	Average	1767	b	25.7	c	151	b	1.0	b
Lentil	CB	1548	b	29.6	ef	135	n.s.	1.0	n.s.
	SV	1789	b	33.6	def	118	n.s.	1.1	n.s.
	SN	1929	ab	25.9	ef	112	n.s.	1.0	n.s.
	Average	1755	b	29.7	c	122	b	1.0	b

Note: Different letters indicate significant differences at $\alpha=0.05$ using Tukey's post hoc test. n.s., not significant.

Table 3.9. ANOVA of richness (Chao 1) and diversity (1/D) of rhizosphere and root endophytic bacteria in canola, wheat, pea and lentil grown in Saskatchewan agricultural fields.

	Chao1		1/D	
Source of variation	Rhizosphere	Root interior	Rhizosphere	Root interior
Rhizo-compartment	<0.0001 ***		<0.0001 ***	
Location	0.01 **	0.4 n.s.	0.3 n.s.	0.1 n.s.
Crop	<0.0001 ***	0.0004 ***	<0.0001 ***	<0.0001 ***
Location*Crop	0.01 **	0.1 n.s.	0.004 **	0.3 n.s.

Note: *, **, ***, significant at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., not significant.

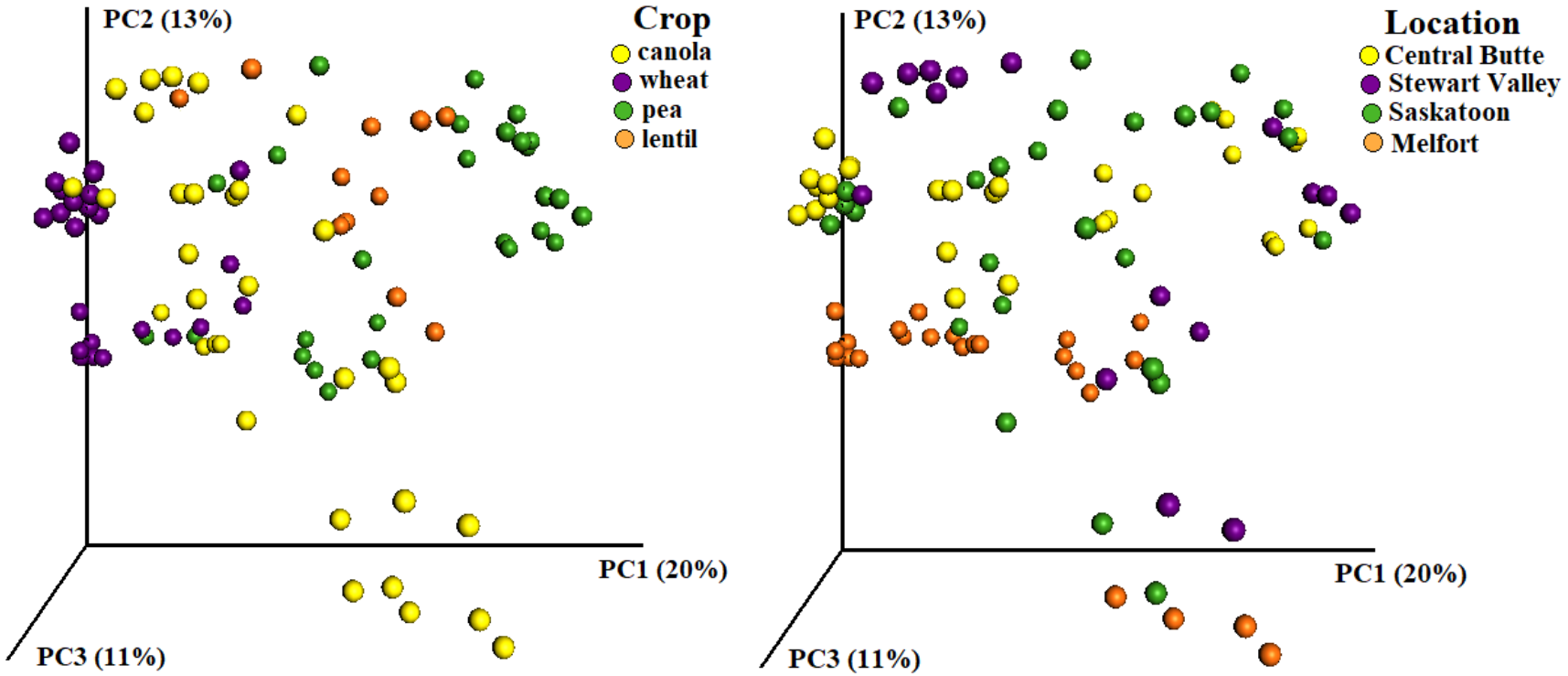

Fig. 3.13. Principal coordinate analysis (PCoA) of rhizosphere bacterial communities based on Bray–Curtis dissimilarity between crops (left) and locations (right).

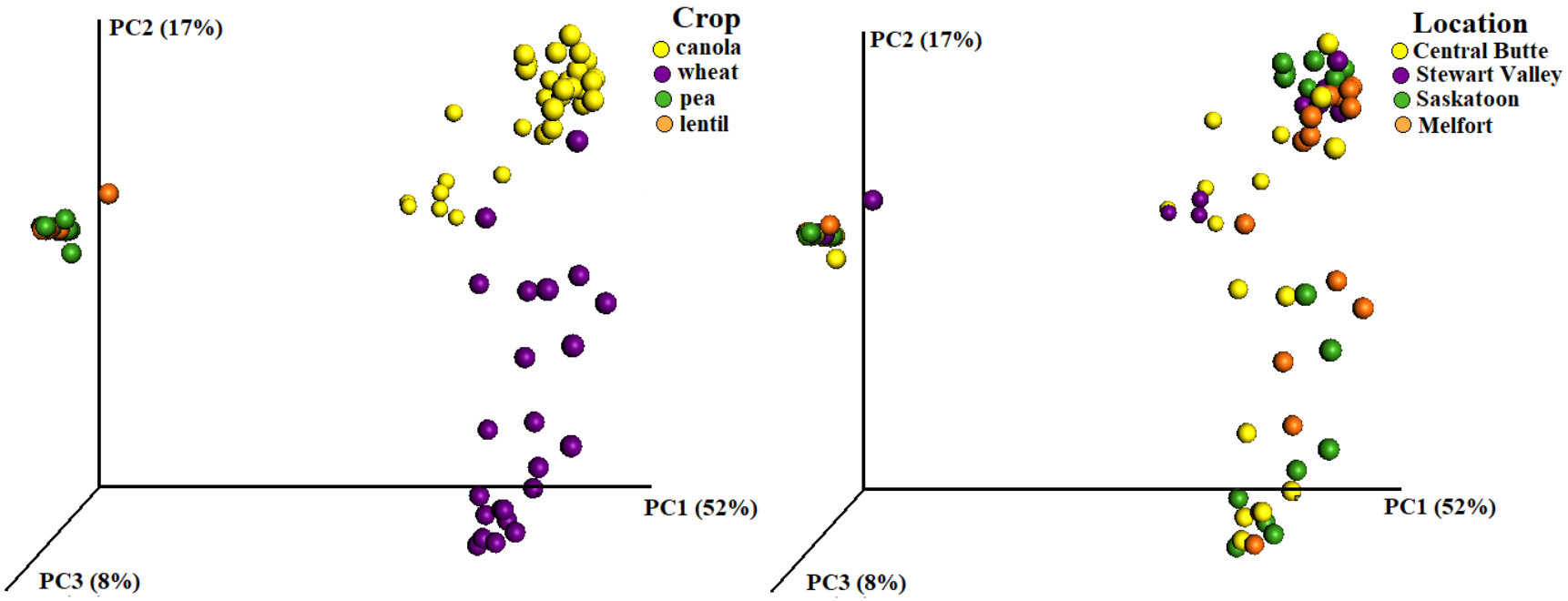

Fig. 3.14. Principal coordinate analysis (PCoA) of root endophytic bacterial communities based on Bray–Curtis dissimilarity between crops (left) and locations (right).

3.5.5. Taxonomic Analysis of Bacterial Communities Associated with Crops assessed by 16S rRNA High-Throughput Sequencing

Phylotype classification of the OTUs resulted in 21 bacterial phyla, from which 20 and 17 phyla were present in the rhizosphere (RZ) and root interior (RI), respectively (Figs. 3.15 and 3.16). Rhizosphere soil associated with the crops exhibited similar phyla profiles, characterized by a high abundance of Proteobacteria and Actinobacteria followed by Bacteroidetes, Gemmatimonadetes, Firmicutes and Acidobacteria. The remaining phyla represented less than 1% of the total OTUs detected in the rhizosphere. However, there were notable differences in the phyla profiles of bacteria colonizing the root interior of the four studied crops. For example, Proteobacteria was the predominant phylum in lentil and pea, followed by canola and wheat. Actinobacteria and Bacteroides were also detected in wheat and canola, whereas their relative abundance was very low in pea and lentil (<0.02%). Relative abundance of the remaining phyla inside the roots was low. Furthermore, the phylum Fusobacteria was only observed in the root interior of canola (Fig. 3.16).

Within the root endophytic Proteobacteria, the genus *Rhizobium* was greatly abundant in the two legume plant species lentil and pea, accounting for 91-99% of the total population. Interestingly, *Rhizobium* was also detected in the interior of wheat and canola roots, accounting for up to 7% of the total endophytic population (Fig. 3.17). Within the rhizosphere, the abundance of *Rhizobium* accounted for up to 38% and 10% in pea and lentil, respectively, opposed to only 2% of the total population detected in wheat and canola. Since the proportion of *Rhizobium* was noticeably inconsistent among the four crops, the genus *Rhizobium* was dropped of the dataset for the analysis of bacterial genera, thus facilitating visualization of rhizosphere and root endophytes community profiles.

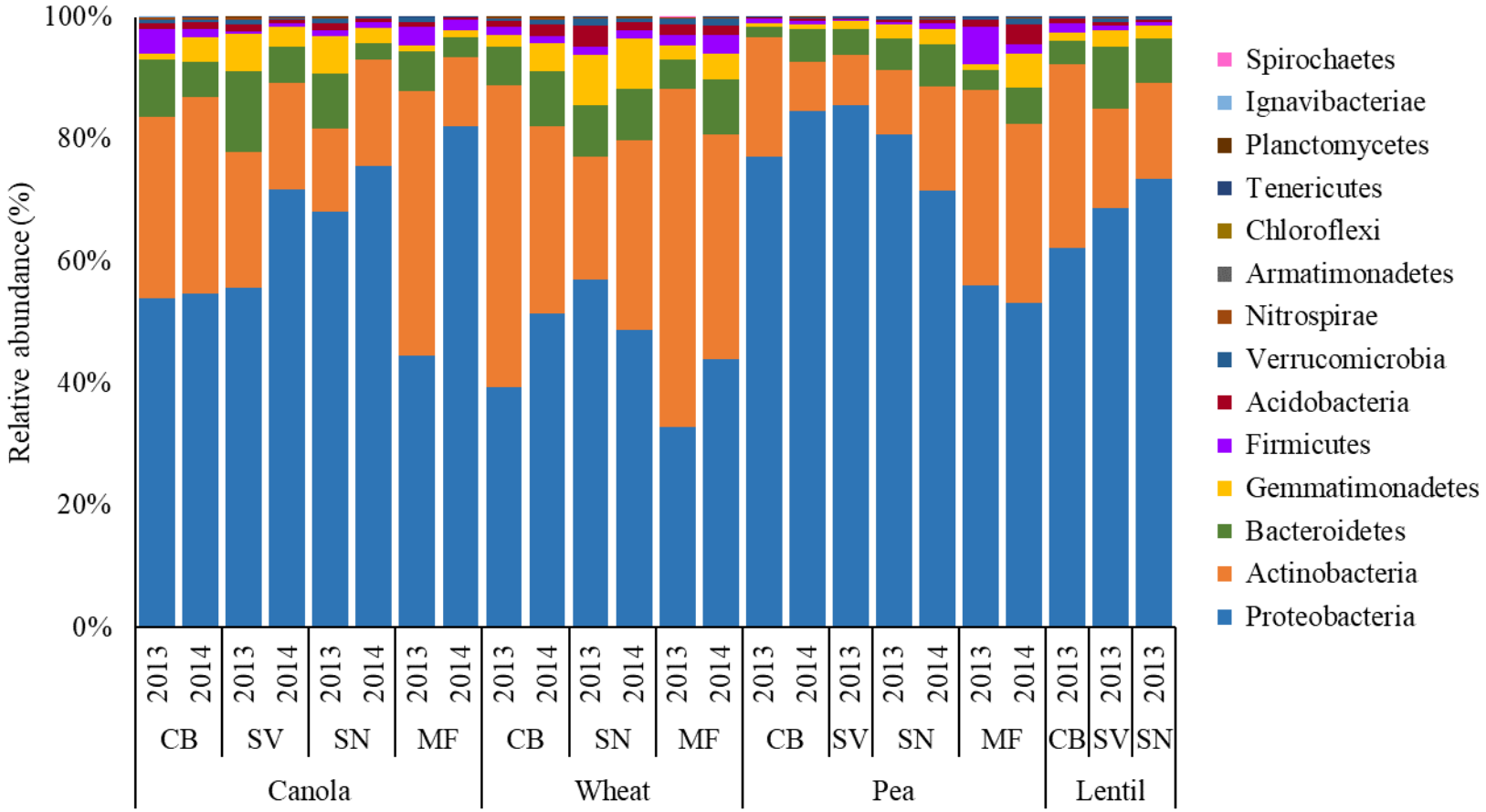

Fig. 3.15. Relative abundance of bacterial phyla level from the rhizosphere of canola, wheat, pea and lentil grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

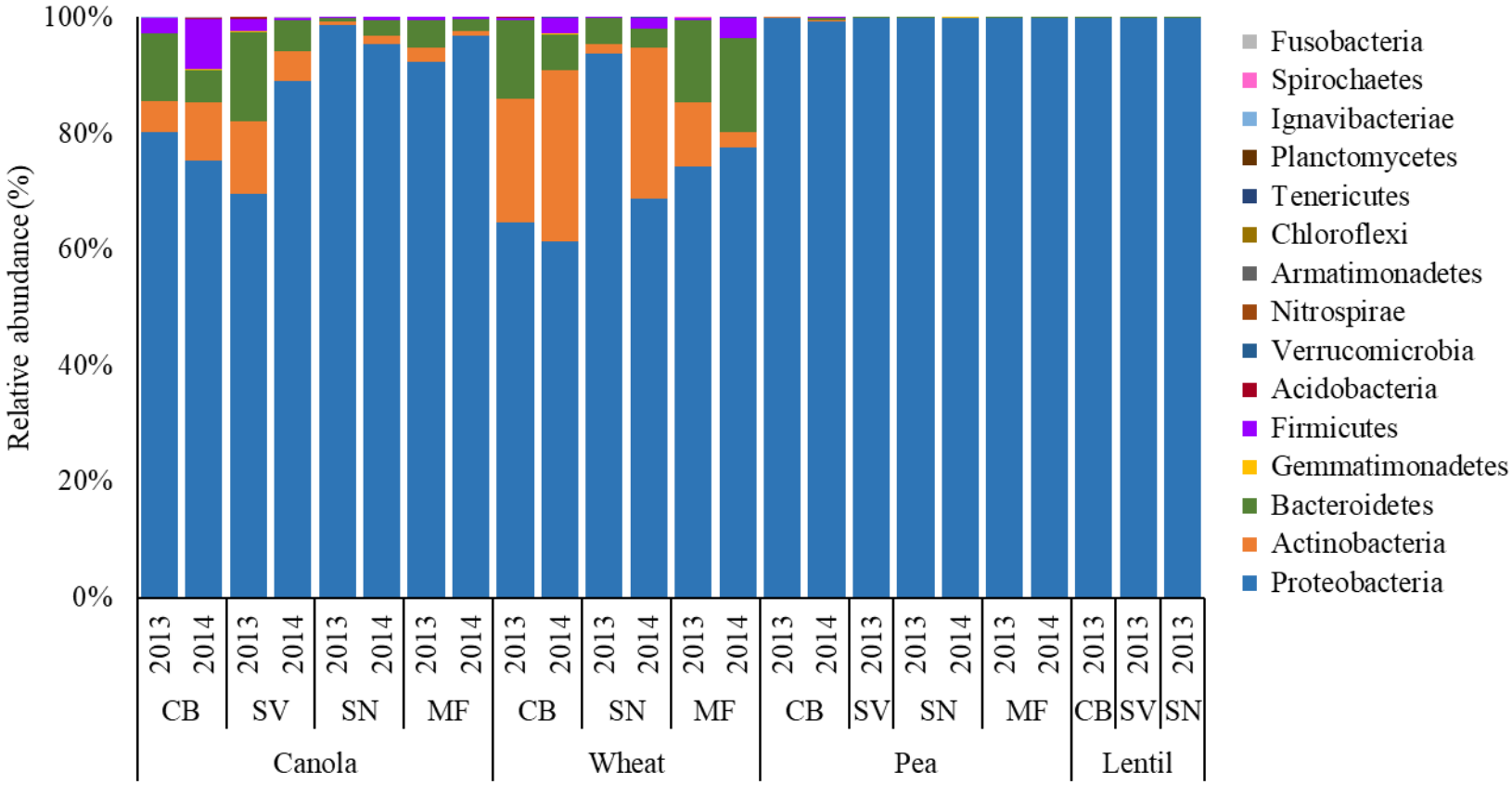

Fig. 3.16. Relative abundance of bacterial endophyte phyla from the roots of canola, wheat pea and lentil collected at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

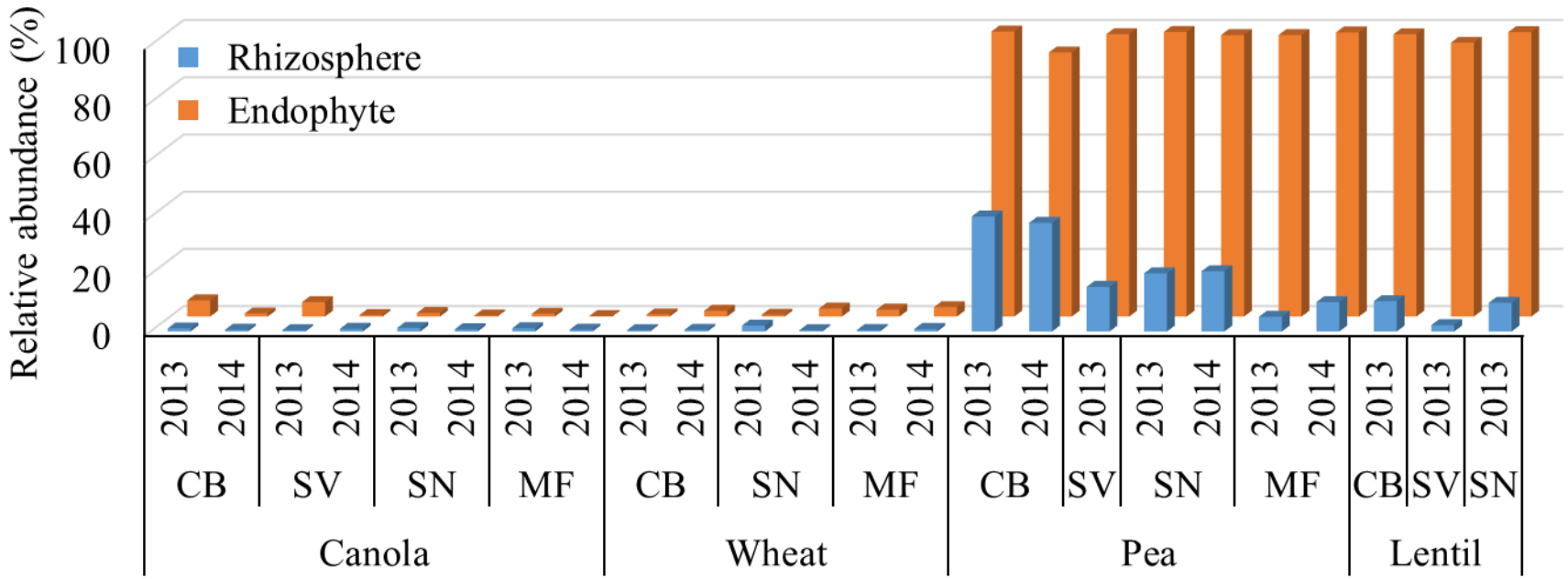

Fig. 3.17. Relative abundance of *Rhizobium* in the rhizosphere and root interior of canola, wheat, pea and lentil grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

Hierarchical clusters based on Bray–Curtis distance of the 0.5% most abundant genera, indicated that the root endophytic bacteria communities grouped in four clusters, which were mainly influenced by crop species (Fig. 3.18). For example, cluster A, which consisted of wheat samples collected at Central Butte in 2013 was characterized by high abundance of *Stenotrophomonas* (45%), as well as *Acinetobacter* (5%) and *Pseudomonas* (5%). In contrast, in cluster B, which also included wheat samples collected in Central Butte, Saskatoon and/or Melfort, consisted mostly of *Pseudomonas* (13%), *Stenotrophomonas* (4%), *Streptomyces* (5%), *Xanthomonas* (5%) and unclassified genera of Enterobacteriaceae (9%). In addition, cluster C, which included only canola samples, exhibited high abundance of *Pseudomonas* (45%), *Stenotrophomonas* (7%), *Acinetobacter* (5%), *Variovorax* (3%) and unclassified genera of Enterobacteriaceae (4%). Finally, in samples collected from pea and lentil (cluster D), unclassified Rhizobiales (32%), Rhizobiaceae (15%), as well as *Pseudomonas* (27%) and *Stenotrophomonas* (7%), *Variovorax* (3%) and unclassified Enterobacteriaceae (5%) were the dominant genera.

Most abundant bacterial genera in the rhizosphere (>1.5%) of all four crops studied grouped into five clusters and appeared to be influenced by locations and crops species, but a definitive trend was unclear (Fig. 3.19). For example, cluster A, which included canola samples collected at Stewart Valley, Saskatoon and Melfort in 2014 consisted mostly of *Stenotrophomonas* (14%), *Acinetobacter* (8%), *Pseudomonas* (3%) and unclassified genera of Enterobacteriaceae (32%). Cluster B, which included lentil and pea samples, as well as canola samples collected at Saskatoon in 2013 mainly consisted of *Pseudomonas* (13%), *Arthrobacter* (4%) as well as unclassified genera of Enterobacteriaceae (6%) and Comamonadaceae (7%). In cluster C which included wheat samples, as well as canola samples collected at Central Butte, Stewart Valley and Melfort, a prevalence of *Gemmatimonas* (5%), *Gaiella* (5%) and unclassified genera of Comamonadaceae (4%) and Rhizobiales (3%) were detected (Fig. 3.19).

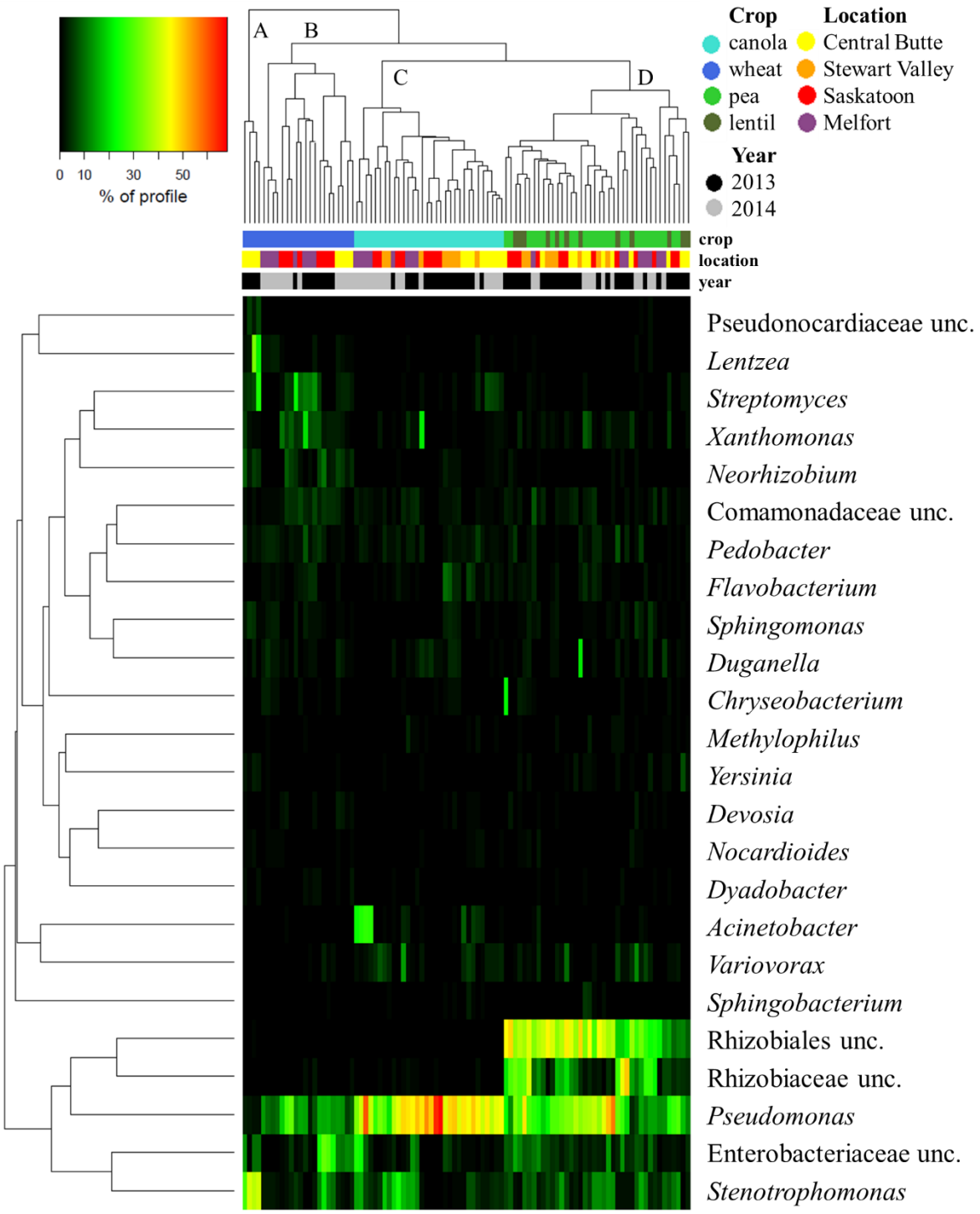

Fig. 3.18. Hierarchical clustering (Bray-Curtis) of bacterial endophyte genera (abundant >0.5%) associated with the roots of canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

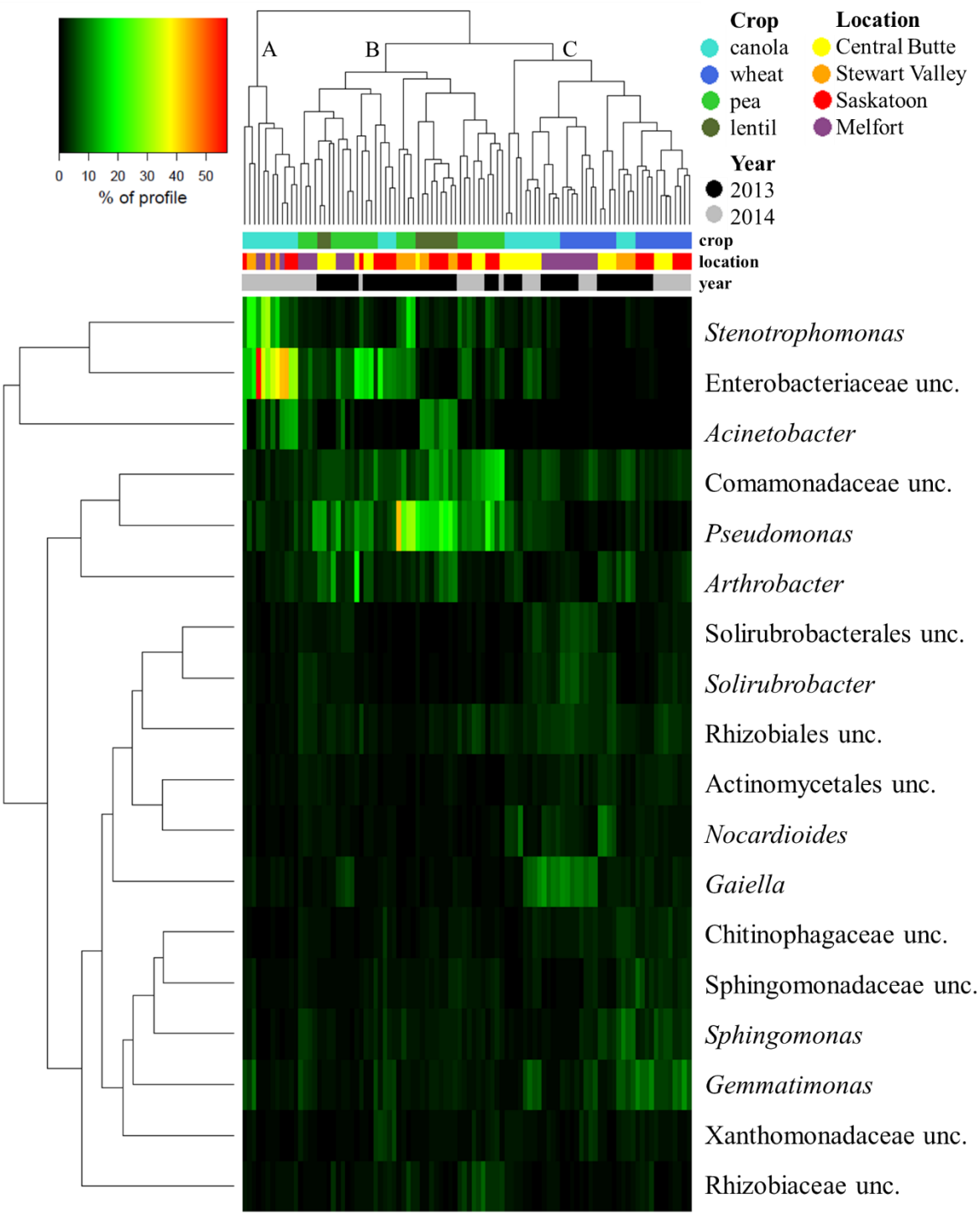

Fig. 3.19. Hierarchical clustering (Bray-Curtis) of bacterial genera (abundant >1.5%) associated with the rhizosphere of canola, wheat, pea and lentil grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

Soil physical and chemical analysis revealed that soil pH, texture and organic matter varied according to locations (Table 3.2). Correlations between soil properties with abundance of bacterial phyla and/or most abundant genera were examined, and only significant values are reported (Table 3.10). In the rhizosphere, abundance of the phylum Firmicutes was positively correlated with the organic matter content. Others *e.g.*, *Bradyrhizobium* and *Gaiella* correlated negatively with soil pH, but positively with the organic matter and silt content. From all sampling locations, the rhizosphere soil from Melfort exhibited the lowest pH and highest organic matter and silt contents and also exhibited a large abundance of Firmicutes, *Bradyrhizobium* and *Gaiella* (Fig. 3.20). No significant correlations were detected between diversity indexes (Chao1 and 1/D) and soil properties in the rhizosphere (data not shown). Conversely, within the root interior, abundance of bacterial genera (>0.5%) and diversity indexes were not significantly correlated with any soil physical characteristics or chemical parameters.

Table 3.10. Pearson correlation coefficients between soil properties (pH, organic matter and silt content) and relative abundance of rhizosphere bacteria associated with canola, wheat, pea and lentil grown in agricultural soils in Saskatchewan.

Classification	pH		OM		silt	
Firmicutes	-0.37	n.s.	0.57	**	0.45	n.s.
Bradyrhizobium	-0.68	***	0.69	***	0.69	***
Gaiella	-0.48	*	0.79	***	0.66	***

Note: *, **, ***, significant at $p \leq 0.05, 0.01, 0.001$, respectively. n.s., not significant.

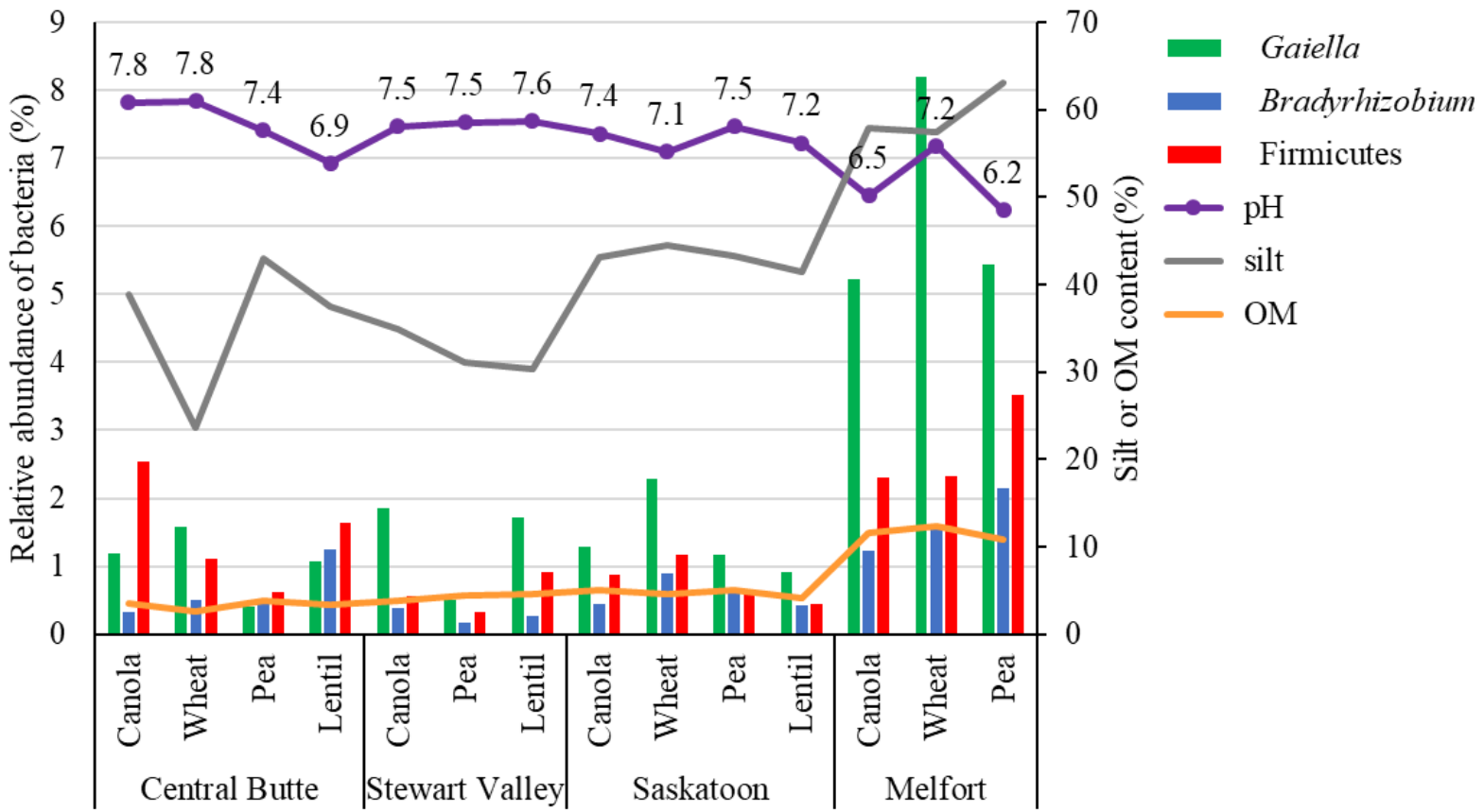

Fig. 3.20. Soil pH, organic matter, silt content and relative abundance of Firmicutes, *Bradyrhizobium* and *Gaiella* associated with crops grown in Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan.

3.6. Discussion

In this study, bacterial communities associated with the rhizosphere and root interior of wheat, canola, pea and lentil were characterized. The number of culturable bacteria was higher in rhizosphere compared to the root interior (Fig. 3.1). Bacterial populations (CFU values) were consistent with previous studies that reported culturable bacterial populations ranging from 10^7-10^9 CFU·g^{-1} fresh root and 10^3-10^7 CFU·g^{-1} fresh root in the rhizosphere and root interior, respectively (Schulz and Boyle, 2006; Compant et al., 2010). In the current study, high-throughput sequencing (Fig. 3.12) and DGGE (Fig. 3.4) analyses suggest that bacterial microbiome of the four crops differed between rhizosphere and root interior. Diversity and species richness of the bacterial communities associated with the various crops suggested that root endophytes were less diverse compared to the rhizosphere bacteria (Table 3.8). These findings suggest that endophytic bacterial communities were a subset of the rhizosphere microbiome (Germida et al., 1998; Bulgarelli et al., 2013; Edwards et al., 2015). Previous studies indicate that during the colonization process, crops may select specific groups of endophytic bacteria by actively changing the composition of roots exudates (Garbeva et al., 2004; Jones et. al., 2009). Root exudates may act as chemo-attractants that mediate the interaction between plant roots and bacteria (el Zahar Haichar et al., 2014). Furthermore, endophytic bacteria may exhibit colonization traits that allow their establishment within the tissues and adaptation to the root environment (Compant et al., 2010). In the current study, results also revealed that only a small portion of the OTUs present in the root interior originate from the rhizosphere; however, these OTUs represented a high percentage of the sequence reads in both, the rhizosphere and root endosphere (Fig. 3.12). Addititionally, some OTUs were found only in the root interior and were not detected in the rhizosphere (Fig. 3.12). These findings suggest that, possibly, these bacterial OTUs colonized the root interior from the rhizosphere in an earlier stage in the crop growing season.

In the current study, the phyla Proteobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Firmicutes and Acidobacteria were the dominant rhizosphere bacteria in the four crops studied (Fig. 3.15). Similar phyla profile was previously reported on a study of rhizosphere communities in wheat and pea (Turner et al., 2013b). Although their results found an enrichment of Proteobacteria, Actinobacteria, Bacteroidetes Firmicutes and Acidobacteria, they also found and Planctomycetes as dominant phylum (Turner et al., 2013b). Similarly, in wheat, the presence of Proteobacteria, Actinobacteria and Bacteroidetes was reported by Donn et al.

(2015). However, in winter wheat, the rhizosphere bacterial communities were enriched with Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Gemmatimonadetes (Mahoney et al., 2017). Other cereal plants are reported to share similar bacterial phyla profile. For instance, DeAngelis et al. (2009) reported that Proteobacteria, Firmicutes and Actinobacteria were the dominant phyla in wild oat, whereas Proteobacteria and Actinobacteria were abundant in rice (Knief et al., 2012). Similarly, Bulgarelli et al. (2015) reported that barley exhibited high abundance of Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Chloroflexi. Canola plants cultivated on agricultural soils from Ottawa, Canada, exhibited some common phyla with our results, such as Proteobacteria, Actinobacteria and Gemmatimonadetes in the rhizosphere (Monreal et al., 2017), whereas winter *Brassica napus* was dominated by Proteobacteria, Actinobacteria and Bacteroidetes (Gkarmiri et al., 2017; Rathore et al., 2017). Other legumes, such as soybean and alfalfa, also exhibited a high abundance of Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria (Xiao et al., 2017).

In the current study, DGGE analyses and high-throughput sequencing revealed that the root interior of the four crops studied was also enriched with Proteobacteria, Actinobacteria and Bacteroidetes (Figs. 3.9 and 3.16). However, contrary to rhizosphere soil, roots exhibited a lower abundance of Gemmatimonadetes and Firmicutes, thus suggesting that crop selection for specific bacterial phyla may have taken place during the root endophytic colonization. Identification of culturable endophytic bacteria using 16S rRNA Sanger sequencing also suggested Proteobacteria and Actinobacteria were the dominant phylum, followed by Firmicutes and Bacteroidetes (Table B.2, Appendix B). The higher abundance of Proteobacteria and Bacteroidetes in the rhizosphere and/or root endosphere, compared to bulk soil, has been attributed to their fast-growing capacity and higher efficiency in metabolizing root exudates (Peifer et al., 2013; Fierer et al., 2007; García-Salamanca et al., 2012). These attributes may allow classifying these Proteobacteria and Bacteroidetes as r-strategists (Peifer et al., 2013; Fierer et al., 2007). In contrast, the phylum Actinobacteria are commonly classified as K-strategists due to their low growth rates and high persistency in soils, even under low nutrient availability (Van Elsas et al., 2006).

When analyzing the four crops for bacterial communities, all crops studied also exhibited distinct phyla profiles. That is, Proteobacteria dominated the root endophytic communities in lentil and pea (mainly represented by *Rhizobium*) (Fig. 3.17). In other legumes, such as red clover, a high dominance of Proteobacteria, *i.e.*, 90% of the total bacterial profile, have been reported

(Hartman et al., 2017), whereas in soybean and alfalfa, Proteobacteria and Actinobacteria have been reported as the most dominant phyla (Xiao et al., 2017). In contrast to pea and lentil, which were dominated mostly by Proteobacteria, in the current study, wheat and canola exhibited a high abundance of Proteobacteria, Actinobacteria and Bacteroidetes (Fig. 3.16). Similarly, Rascovan et al. (2016) reported a high abundance Proteobacteria, Firmicutes, Planctomycetes and Verrucomicrobia in the root interior of wheat, whereas Ofek et al. (2014) only detected Proteobacteria and Actinobacteria as the dominant phyla in wheat. Additionally, bacterial endophytes associated with durum wheat were dominated by Proteobacteria, Firmicutes and Actinobacteria (Yang et al., 2012). Other cereals, such as barley, exhibited high abundance of Proteobacteria, Bacteroidetes and Actinobacteria (Bulgarelli et al., 2015), whereas rice plants were enriched with Proteobacteria and Firmicutes (Sessitsch et al., 2012). Similarly, studies conducted by de Campos et al. (2012), Gkarmiri et al. (2017) and Rathore et al. (2017) demonstrated that the root interior of winter *Brassica napus* and canola also comprised of a high abundance of Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. Interestingly in the current study, the phylum Fusobacteria, represented by *Fusobacterium*, was only detected in the root interior of the canola. Fusobacteria are human pathogens, obligate anaerobes and non-spore forming G-bacilli (Bennet and Eley, 1993). The phylum Fusobacteria also has been found in the roots of winter *Brassica napus* and potato (Manter et al., 2010; Gkarmiri et al., 2017). The presence of *Fusobacterium* in the root interior, but its absence in the rhizosphere, suggest that *Fusobacterium* may have been horizontally transmitted to the roots from aboveground plant canola organs, and/or vertically transmitted from the seeds.

Analysis of community structure using DGGE and 16S rRNA high-throughput sequencing, indicated that root bacterial endophytes were mainly influenced by the host crop (Figs. 3.8 and 3.14), whereas the rhizosphere bacterial communities varied among crop species and/or sampling locations (Figs. 3.10 and 3.13). Similarly, species richness (Chao1) of endophytic bacteria differed among crops but did not differ among locations. Rhizosphere bacteria were influenced predominantly by the interaction of crop and locations, as indicated by Chao1 and 1/D (Table 3.8). The lack of correlation between the most abundant bacterial endophyte genera and soil physical and chemical parameters (Table 3.10) suggests that root endophytes may be influenced by factors related to the host plant rather than soil characteristics. Bulgarelli et al. (2013) suggested that the selection of root microbiota from the bulk soil was mediated by the release of root exudates in the

rhizosphere. On a second stage, endophytic bacterial communities present in the rhizosphere were selected to enter the root interior by the host crop during the colonization (Bulgarelli et al., 2013). In addition, plant species and/or cultivars, plant growth stage, and plant health also are known to influence the interactions that occur between host crop and bacterial endophytes (Garbeva et al., 2004). Additional factors affecting the selection of specific bacterial endophytes include differences in root morphology, composition of root exudates or the presence of wounds that may favor the penetration of bacteria into the host plant roots (Gaiero et al., 2013).

As indicated by DGGE analysis, *Pseudomonas* was a dominant genus in the root interior of wheat and canola (Fig. 3.9). However, in addition to *Pseudomonas*, high-throughput sequencing analysis also revealed that *Stenotrophomonas* was highly abundant in the rhizosphere soil and root interior of canola, wheat, pea and lentil (Figs. 3.18 and 3.19). Similarly, culture dependent methods detected a high number of *Pseudomonas* associated with canola and lentil roots, whereas *Stenotrophomonas* was also detected in canola (Table A.2, Appendix A). These results suggest that *Pseudomonas* and *Stenotrophomonas* were widely distributed in all four soils studied, hence their high abundance in all the four studied crops. Other studies assessing culturable bacteria associated with canola roots also detected *Pseudomonas* as a common genus associated with plants collected in agricultural soils from Belgium, Brazil, Canada, China France and Scotland (Bertrand et al., 2001; Misko and Germida, 2002; Farina et al., 2012; Croes et al., 2013; Rathore et al., 2017; Zhao et al., 2017). Studies on wheat root endophytes in Argentina, Canada and Israel, also detected *Pseudomonas* as the dominant genera (Germida and Siciliano, 2001; Rascovan et al., 2016; Ofek et al., 2014). Other authors also reported *Pseudomonas* as a predominant genus in the rhizosphere of wheat and canola (Ofek et al., 2014; Croes et al., 2013; Donn et al., 2015). Additionally, *Stenotrophomonas* also was reported in canola grown in agricultural soils in Brazil (de Campos et al., 2012). Similarly, *Pseudomonas* and *Stenotrophomonas* are reported as the most abundant genera in the root interior of legumes such as red clover (Hartman et al., 2017). Bacterial endophytes, such as *Pseudomonas* and *Stenotrophomonas* are often deemed as generalists and potentially they can impart beneficial properties to numerous plant species (Compant et al., 2005; Khan et al., 2012). For instance, Alstrom (2001) and Farina et al. (2012) reported that *Pseudomonas* spp. isolated from canola produced indole compounds and siderophores, solubilized phosphorus and exhibited biocontrol activity against pathogenic fungi. Similarly, pseudomonads have been demonstrated to produce antiobiotic, stimulate wheat growth and increase root dry

weigh of canola (Thomashow et al., 1990; Germida and Walley, 1996; Bertrand et al., 2001). *Stenotrophomonas maltophilia* is reported to increase resistance against biotic and abiotic stress in wheat, whereas it has been demonstrated to stimulate plant growth in canola (Banerjee, 1995; Singh and Jha, 2017). Generalist bacterial endophytes are hypothesized to be horizontally transmitted since they can be found in a variety of plant species. Frank (2017) reported that generalist endophytes distribution is more correlated to environmental factors rather than to the host plant.

Although *Pseudomonas* and *Stenotrophomonas* were associated with all four crops studied, additional bacterial genera were predominant only in certain crops, suggesting that selection of a bacterial consortia associated with the root interior of canola, wheat, pea and lentil, was crop-specific. For example, in canola, high-throughput sequencing detected high abundance of *Variovorax* (Fig. 3.18), whereas *Streptomyces*, *Microbacterium*, *Bacillus* and *Acinetobacter* were the most common culturable genera (Table A.2, Appendix A). Similar studies, have reported a high abundance of *Variovorax* in the rhizosphere and root interior of canola plants (Croes et al., 2013; de Campos et al., 2012). In addition, *Variovorax* was reported to stimulate root dry weigh of canola, whereas *Acinetobacter* and *Bacillus* stimulated root growth and exhibited biocontrol activity against *Sclerotinia sclerotiorum* (Bertrand et al., 2001; Indiragandhi et al., 2008; Fernando et al., 2007; Ramarathnam et al., 2007).

In wheat, high-throughput sequencing analysis and culture dependent methods revealed a predominance of *Xanthomonas* (Table A.2, Appendix A and Fig. 3.18). *Xanthomonas* was also reported in several wheat cultivars grown in Saskatchewan (Germida and Siciliano, 2001). In the current study, high-throughput sequencing and DGGE demonstrated that wheat plants also harbored a high abundance of *Streptomyces* and *Arthrobacter*, respectively (Figs. 3.9 and 3.18). Culture dependent methods revealed that, *Mycobacterium*, *Brevibacillus* and *Erwinia* were also prevalent genera in wheat (Table A.2, Appendix A). Previous studies assessing culturable endophytic Actinobacteria in wheat roots grown in Australia also detected *Arthrobacter*, *Streptomyces* and *Mycobacterium* among the most abundant genera (Coombs and Franco, 2003; Conn and Franco, 2004). Similarly, Kumar et al. (2014) also have reported that an *Arthrobacter* sp. was able to fix N_2, solubilize phosphate and promote wheat growth in a growth chamber and field conditions.

As expected, culture dependent methods, DGGE analysis and high-throughput sequencing confirmed that *Rhizobium* was the most dominant genus in pea and lentil (Table A.2, Appendix A, Figs. 3.9 and 3.17). Symbiosis between rhizobia and legumes have been extensively investigated because of their key role on the evolution and ecology of leguminous plants such as pea and lentil (Lindstrom et al., 2010; Masson-Boivin et al., 2009). Interestingly in the current study, the genus *Rhizobium* was also detected in wheat and canola roots, which accounted for 2% of the total bacterial population (Fig. 3.17). Endophytic rhizobia have been previously reported in wheat and canola (Lupwayi et al., 2004). Although there was no conclusive evidence that this association resulted in symbiotic nitrogen fixation, Sharma et al. (2005) identified a *Rhizobium* in the root interior of wheat plants, which produced indole acetic acid and increased seedling shoot and root length. In addition to some *Rhizobium*, in the current study, culture dependent methods also indicated a high prevalence of *Bacillus* and *Microbacterium* in lentil and pea. Similarly, the genus *Bacillus* was reported to be a common bacterial endophyte in other legumes such as red clover and peanut (Wang et al., 2013; Hartman et al., 2017), and *Microbacterium* was isolated from the nodules of the legume *Lespedeza* sp. (Palaniappan et al., 2010).

Despite the presence of distinct genera profiles in the root interior of each crop studied, the rhizosphere bacterial microbiome associated with canola, wheat, pea and lentil varied greatly among crops and sampling locations (Figs. 3.1, 3.10, 3.13 and Table 3.8). This result suggests that soil properties may have influenced the diversity of rhizosphere bacteria in the crops. For example, abundance of Firmicutes, *Bradyrhizobium* and *Gaiella*, in the rhizosphere was significantly correlated with soil pH, silt and organic matter content across sampling locations (Table 3.10, Fig. 3.20). Similarly, the total PLFAs biomass (nmol·g^{-1} soil) including bacterial, G+ and G- in the soil was positively correlated with soil organic matter and silt content, but negatively correlated with soil pH (Fig. 3.2). Previous studies conducted by Garbeva et al. (2004) indicated that soil properties can influence bacterial communities composition, not only in the bulk soil, but also communities in the rhizosphere. Soils have the ability to provide specific habitats for distinct groups of microorganisms, or to affect the physiology of the plant root, which indirectly influences the rhizosphere microbiome (Garbeva et al., 2004). Soil texture is known to influence bacterial community structure mainly by modulating water content and movement within the soil matrix (Carson et al., 2010). Other authors have reported that soil pH is the major factor determining bacterial diversity and the phyla composition in different soils including agricultural fields in

Canada, in soils from across North and South America, Great Britain, and in polar soil ecosystems (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011; Li et al., 2012; Siciliano et al., 2014). Lauber et al. (2009) suggested that soil pH affects bacterial communities directly by imposing a physiological limitation for bacteria in the soil, which may modulate competition among species or alter the dominance of certain taxa. Alternatively, Lauber et al. (2009) reported that soil pH influences nutrient availability, salinity and organic carbon, thus regulating the physiology of the microbial community in the soil. In agreement, studies conducted by Ferguson et al. (2013), indicated that *Bradyrhizobium* spp. are generally tolerant to acidic conditions in soil, which may explain their higher abundance in locations with lower soil pH in the current study (Fig. 3.20). Similarly, Albuquerque et al. (2011) reported that *Gaiella* have optimal pH for growth between 6.5 and 7.5, which suggests that the abundance of *Gaiella* in the rhizosphere also may be influenced by the soil pH levels. The genus *Gaiella* was previously reported as a prevalent bacterial group in the rhizosphere of canola and legumes (Monreal et al., 2017; Xiao et al., 2017). In the current study, the higher abundance of Firmicutes in the rhizosphere of crops grown at Melfort, *i.e.*, a location with high organic matter content, may be related with the rapid growth of this phylum on readily available carbon sources (Van Elsas et al., 2006). In addition, Firmicutes can survive in the soil for prolonged periods of time due to their capacity to form endospores (Van Elsas et al., 2006).

3.7. Conclusions

Analysis of bacterial communities associated with canola, wheat, pea and lentil using high-throughput sequencing, DGGE and culture dependent methods revealed that crops selected specific bacterial consortia within their roots. Endophytic bacterial communities consisted mainly of phyla Proteobacteria, Actinobacteria and Bacteroidetes. At the genera level, distinct endophytic bacterial profiles were associated with the root interior of each crop studied, whereas the rhizosphere bacterial communities greatly varied among crop species and sampling locations. Several of the identified bacterial genera were previously reported to play important roles as plant growth promoters. Furthermore, soil properties such as pH, silt and organic matter content influenced bacterial populations in the bulk soil as well as in the rhizosphere of the four crops studied.

4. ASSESSMENT OF BACTERIAL MICROBIOMES OF WHEAT AND CANOLA AT DIFFERENT PLANT GROWTH DEVELOPMENT STAGES

4.1. Preface

Agricultural crops establish relationships with bacteria, which can result in plant health promotion and increased crop productivity. However, plant developmental processes may influence the diversity and function of the associated bacterial communities. Much is known about plant-bacteria interactions in the rhizosphere, but it is important to understand how these interactions may influence the whole plant microbiome. In this chapter, the bacterial microbiome associated with the rhizosphere, roots, shoots and seeds of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) at stem elongation, flowering and ripening stages was assessed using culture independent techniques *e.g.*, Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rRNA high-throughput sequencing.

4.2. Abstract

Agricultural crops can establish neutral, deleterious and beneficial relationships with soil bacteria. Beneficial relationships have the potential to promote crop health and increased productivity. However, during various plant developmental processes a shift in the diversity and function of bacterial communities often occurs. Plant × bacteria interactions in the rhizosphere are well known; however, it is important to understand how plant × microbe interactions may influence the whole plant microbiome. This study investigated the diversity of bacterial communities associated with wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) at stem elongation, flowering and ripening stages. Wheat and canola were grown in Brown and Black Chernozem soils in a growth chamber. The soils were collected from agricultural fields in Saskatchewan, Canada and differed mainly in organic matter content, pH and texture. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of 16S rRNA gene amplicons revealed that bacterial communities associated with wheat and canola exhibited distinct DGGE band profiles among the rhizosphere, root, shoot and seed of both crops. Bacterial community profiles of each plant species were similar at stem elongation and flowering stages; however, a shift in community structure was observed at ripening of both crops. Phylogenetic analysis of the bacterial microbiome using 16S rRNA high-throughput sequencing revealed that crop species and soils were the main factors affecting the community structure of rhizosphere and root endophytic bacteria, whereas, the aboveground plant compartments exhibited high variability in the bacteria community profiles. These results also suggest that plant growth stages can modulate the diversity of rhizosphere and endophytic bacterial communities and that the influence of plant growth stages on the bacterial microbiome associated with wheat and canola was crop and organ specific.

4.3. Introduction

The microbiome associated with plants includes all microorganisms inhabiting external surfaces and internal organs. Due to their relevance to plant growth and development the microbiome is often considered as the second plant's genome. Among these microorganisms, bacteria are important in promoting plant growth and health, and/or improving the sustainability of crop production (Berg et al., 2014). For example, crop associated bacteria may have the potential to stimulate plant growth by several mechanisms including the production of plant growth regulators; suppression of abiotic stress; biological nitrogen fixation; and/or phosphorus mobilization (Ali et al., 2014; Compant et al., 2010). In addition, bacteria may share similar habitats as phytopathogens, thus making it possible to control the spread of potential pathogens. Such biological control activity is mainly through: (*i*) inducing plant defence mechanisms; (*ii*) producing pathogen-antagonistic substances, and/or (*iii*) competing with pathogens for plant colonization sites and nutrients (Reinhold-Hurek and Hurek, 2011).

Bacterial communities colonize plant tissues mainly using two pathways including: horizontally *i.e.*, entering the plant tissues from the environment, or vertically *i.e.*, transmitted from the parent plant to its offspring through seeds (Bright and Bulgheresi, 2010). Bacterial colonization of crops begins at seed germination stage, in which seed exudates attract bacteria from soil into the spermosphere (Nelson, 2004). Additionally, during further growth of crops, plant root exudates also are important in shaping the microbiome in the rhizosphere, the rhizoplane and subsequently the root interior. After entering roots, bacterial endophytes may translocate into the xylem vessels and eventually colonize aboveground plant tissues (Compant et al., 2010). Bacteria may also colonize above-ground surfaces and penetrate plant organs such as stem, leaves, flowers and fruits. Potential sources of bacteria for aboveground plant colonization includes the atmosphere, rain, or pollinators (Frank et al., 2017). In addition, vertical transmission of bacteria through seeds has been described as the main pathway for the colonization of most obligate endophytes, which depend strictly on the host plant for their growth and survival (Hardoim et al., 2008). Despite the wide distribution of bacteria within host plants, most of the research related to crop associated bacteria is focused on the rhizosphere. In contrast, there are few reports on bacteria colonizing above-ground tissues such as stems, leaves and seeds (Sessitsch et al., 2002; Coombs and Franco, 2003; Hardoim et al., 2012).

Previous studies have reported that bacterial communities associated with crops are strongly influenced by plants species and/or cultivar (Germida et al., 1998; el Zahar Haichar et al., 2008; Ofek-Lalzar et al., 2014). For example, the selection of rhizosphere microbiota from the bulk soil is modulated by the release of root exudates by the crops (Bulgarelli et al., 2013). Bacterial penetration into the root interior is also influenced by the morphology of the root system as bacteria colonization occur mainly by accessing root tips and/or epidermis discontinuities, *e.g.*, lateral root emergence sites or wounds (Gaiero et al., 2013). Bacterial multiplication within internal plant tissues also involves the recognition of plant signals that may induce cellular processes necessary for the establishment of endophytes inside the host crop (Hardoim et al., 2008). Aerial vegetative organs are colonized by fewer species of bacterial endophytes; however, these bacterial communities are well adapted to specific plant niches in the aboveground plant organs (Hallmann, 2001). Although there is a close interaction between the host plant and their microbiome, it remains unclear if the diversity of endophytes associated with crops differs among plant compartments (Compant et al., 2010).

The physiological processes occurring during plant growth may influence the bacterial microbiome of crops. Previous studies report that rhizosphere bacterial communities associated with Arabidopsis, Medicago, maize, pea, wheat, canola and sugar beet, change during plant developmental stages (Baudoin et al., 2002; Dunfield and Germida, 2003; Mougel et al., 2006; Houlden et al., 2008; Micallef et al., 2009). These studies suggest that changes in the root exudation pattern, which occur as plants develop, may alter rhizosphere microbial community composition. However, studies analysing the influence of growth development stages on the bacterial microbiome associated with both below and aboveground plant compartments are scarce. In this study, it was hypothesized that bacteria associated with wheat and canola grown in agricultural soils will differ between plant organs and growth stages. The main objective of this study was to assess the diversity and relative abundance of bacteria colonizing the rhizosphere, roots, shoots and seeds of wheat and canola plants at stem elongation, flowering and ripening stages.

4.4. Materials and methods

4.4.1. Experimental Design

Soils were collected from wheat fields at Central Butte (50°43'N, 106°25'W) and Melfort (52°49'N, 104°36'W), corresponding to Brown and Black Chernozems, respectively (Soil Classification Working Group, 1998). Soil samples were sent to ALS Environmental Laboratory (Saskatoon, Saskatchewan) for basic soil analysis (Table 4.1). Protocols used for soil analysis were described previously in section 3.4.2 (chapter 3). Bulk soil was air dried, sieved (<2mm) and 1.5 kg transferred to 1.5 L plastic pots. Wheat (CDC Waskeda) and canola (Invigor L150) were grown in potted soils (n=4). Ten wheat or canola seeds were placed in the soil, allowed to germinate, and then thinned so only 2 plants were left to grow. Plants were grown in a growth chamber with a 16 h/25°C day (1500 $\mu mol \cdot m^{-2}$) and 8 h/15°C night cycle. The soil was moistened with sterile distilled water and maintained at 50% moisture holding capacity throughout the experiment. Plants were harvested at stem elongation, flowering and ripening stages (Figs. C.1-C.6, Appendix C) following a universal growth stage scale (Lancashire et al., 1991).

Table 4.1. Physical and chemical properties of Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

						Available			
	pH	Sand	Silt	Clay	OM	NO_3^-	SO_4^{2-}	PO_4^{3-}	K^+
		%				($mg \cdot g^{-1}$ soil)			
Central Butte	7.6	65	25	10	3.1	5.5	6.6	28.6	584
Melfort	6.1	15	55	30	14.6	15.9	7.1	10.7	624

4.4.2. Sampling of Rhizosphere Soil, Root, Stem, Leaf and Seed

At each sampling time, aboveground plant organs were separated from the roots using aseptic techniques. Plant roots (2 g) with adhering soil were placed in a 500 mL Erlenmeyer flask containing 200 mL of sterile PBS and placed on a rotary shaker (150 rpm) at 22°C for 25 min. Then, resulting soil slurry was transferred to 50 mL Falcon tubes and centrifuged (2000 × g for 5 minutes). The supernatant containing PBS buffer was discarded and the rhizosphere soil stored at -80°C for DNA extraction (Dunfield and Germida, 2003). Approximately 5 g of each plant organ were washed with sterile tap water to eliminate soil and particles from the plant surface. Then, a

1g portion of plant material was placed into a 300 ml Erlenmeyer flask containing 100 ml NaClO (1.05% $v{\cdot}v^{-1}$) in sterile PBS and placed on a rotary shaker (150 rpm) at 22°C for 15 min (Siciliano and Germida, 1999). Next, surface disinfected plant material was cut into 0.5 cm portions using a sterile scalpel and stored at -80°C for molecular analyses. Furthermore, surface disinfection of collected seeds was accomplished by submerging the seeds in 65% ($v{\cdot}v^{-1}$) ethanol for 3 min, then submerging for 5 min in NaClO (1.2% $v{\cdot}v^{-1}$) and finally rinsed 10 times in sterile tap water (Vincent, 1970). Surface sterilization of plant material was checked by spreading 0.1 ml of the last wash onto 1/10th strength tryptone soy (1/10 TSA) solidified with 1.5% agar.

4.4.3. DNA Extraction

Total genomic DNA was extracted from rhizosphere soil and plant material using soil and plant DNA extraction kits (MO BIO Laboratories, Inc.), respectively. The DNA extractions were conducted following the manufacture's protocols. The DNA yield was quantified using Qubit DNA HS Assay Kit (Thermo Fisher Scientific) and DNA electrophoresis in 1% agarose gels stained with the SYBR™ safe DNA gel stain (Invitrogen).

4.4.4. Analysis of Bacterial Communities using Denaturing Gradient Gel Electrophoresis (DGGE)

Community structure of the rhizosphere soil and plant endophytic bacteria was examined by DGGE. The amplification of 16S rRNA gene was performed using the primer pair U341 (with GC-clamp) (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG_CCT ACG GGA GGC AGC AG-3') and U758 (5'-CTACCAGGGTATCTAATCC-3') (Phillips et al., 2006). PCRs were performed in a reaction volume of 50 µL consisting of 1 µL of DNA extract, 1 µL each primer (25 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.63 µL BSA (10 $mg{\cdot}mL^{-1}$) (Bovine serum albumin, Amersham Biosciences, Mississauga, ON, Canada), 25 µL of Hot Star Master Mix and 21.37 µL of RNase-free water (Qiagen, Toronto, Ontario, Canada). Amplifications were performed for 10 cycles of 1 min denaturing at 94ºC, 1 min annealing at 65-55ºC and 1 min extension at 72ºC. This was followed by 20 cycles using an annealing temperature of 55ºC. Touchdown PCR was used in the annealing step to minimize nonspecific priming. Amplification fragments sized about 417 bp were confirmed by electrophoresis on 1.5% agarose gels in 1×TBE buffer containing the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada).

The PCR amplification products were analyzed using DGGE for the visualization of DNA bands representing dominant bacterial species (Muyzer et al., 1993). Briefly, amplicon aliquots were loaded onto an 8% acrylamide gel with a 40-60% denaturing gradient. Electrophoresis was performed for 16h at 80V and 60°C, and the resulting gels were stained with the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada). Random dominant bands were excised from the gel using a sterile scalpel, vortexed briefly in 60 µL of TE buffer, eluted for 30 min at 37ºC and centrifuged at 10000 × g for 1 min at room temperature. Then, DNA was re-amplified using the primers U341 (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG-3') and U758 (5'-CTACCAGGGTATCTAATCC-3') (Phillips et al., 2006). PCRs were performed in a reaction volume of 50 µL consisting of 1 µL of DNA extract, 1 µL each primer (25 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.63 µL BSA (10 mg·mL^{-1}) (Bovine serum albumin, Amersham Biosciences, Mississauga, ON, Canada), 25 µL of Hot Star Master Mix and 21.37 µL of RNase-free water (Qiagen, Toronto, Ontario, Canada). Amplifications were performed for 25 cycles of 1 min denaturing at 94ºC, 1 min annealing at 64ºC and 1 min extension at 72ºC. PCR Amplified fragments were run on 1.5% agarose gels in 1×TBE buffer containing the SYBR™ safe DNA gel stain (Invitrogen) and visualized using a Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, Mississauga, ON, Canada). Finally, PCR amplicons were sequenced by Macrogen Inc. (Seoul-Rep. of Korea). Bacteria were identified by comparison of DNA sequences in GenBank databases using the BLAST algorithm (Altchul et al., 1997).

4.4.5. Analysis of Bacterial Communities using 16S rRNA High-Throughput Sequencing

The DNA samples were submitted for high-throughput sequencing to the Génome Québec Innovation Centre, McGill University using Illumina technology. PCR amplifications were conducted using the primers 520F (5`-AGCAGCCGCGGTAAT-3`) and 799R2 (5`-CAGGGTATCTAATCCTGTT-3`) that amplifies the V4 region of the 16S rRNA gene (Edwards et al., 2007). Sample libraries were prepared according to the MiSeq reagent kit preparation guide (Illumina, San Diego, CA), and the sequencing protocol from Caporaso et al. (2010).

4.4.6. Bioinformatics and Statistical Analyses

The DGGE gel analysis, band detection and cluster analysis were performed using Bionumerics version 5.1 (Copyright © 1998 Applied Maths, Austin, TX). Selection of DGGE

bands was done using a minimum profiling, position tolerance and optimization of 5%, 1.5% and 2%, respectively. Densitometric curves were used to perform band matching, creating a binary presence-absence matrix (Boon et al., 2002; Peixoto et al., 2006). The influence of crop, soil, plant compartment and development stages on the bacterial community profile based on DGGE were analyzed by non-metric multidimensional scaling (NMDS) using PCOrd software (McCune and Grace, 2002) with the Autopilot Slow and Thorough analysis options. The statistical significance of the final solutions was determined by comparing the final stress values among the best solution for each axis using the Monte Carlo test. The final stress value indicated the reliability of the final ordination in relation to the dataset dissimilarities. Final stress values obtained in this study ranged between 10 and 20, which indicate an acceptable ordination with no real risk of misinterpretation for most ecological community datasets. A multi-response permutation procedure (MRPP) was performed using the Sorensen distance measure to test for differences between groups. The chance-corrected within-group agreement index (A) was a proportion between heterogeneity within groups in relation to heterogeneity expected by chance. Thus, A indicated accuracy of the clustering within samples of the same group. Values close to zero indicated herogeneity within the group equal expectation by chance, whereas values close to 1 indicated that all samples within the groups were identical.

Sequences derived from rhizosphere and plant endophytic bacteria using high-throughput Illumina technology were analyzed using Mothur v.1.34.3 (Kozich et al., 2013). The standard operating procedure included the generation of contigs from the combination of forward and reverse reads and the removal of sequence errors and chimeras. Sequences from chloroplasts, archaea, eukaryotic organisms were also removed. Taxonomic classification was done with naive Bayesian classifier using SILVA database. Operational taxonomic units (OTU) numbers were calculated at a distance 0.03 (97% similarity). Relative abundance of a bacterial taxa in a sample was calculated as the percentage of sequence reads belonging to the bacterial taxa relation to the total number of reads in a sample. Rarefaction curves values, Simpson's reciprocal (1/D) diversity and Chao 1 richness were generated using Mothur software at OTU cutoffs of 0.03 distance units using the number of observed OTUs. The influence of crop, soil, plant compartment and development stages on the bacteria OTU distribution was analyzed by Principal Coordinate Analysis (PCoA) using QIIME (Quantitative Insights Into Microbial Ecology) 1.9.1 (Caporaso et al., 2010). Heatmaps of the most abundant genera (relative abundance higher than 0.5%) were

conducted using the VEGAN package (version 2.0–7) in R version 2.15.2 (R Core team, 2012). The richness and diversity indexes were subjected to analysis of variance (ANOVA) and Tukey's post hoc test using SAS software version 9.6 (Copyright © 2002-2010 SAS Institute Inc. Cary, NC, USA). The sequence data can be accessed in NCBI under Genome Project ID 510722 (accession PRJNA510722).

4.5. Results

4.5.1. DGGE Profiles of Rhizosphere Soil, Root, Stem, Leaf and Seed Endophytic Bacteria

Assessment of community structure of rhizosphere and plant associated bacteria in wheat and canola using DGGE indicated distinct population profiles amongst plant compartments and growth stages (Figs. 4.1 and 4.2). Non-metric multi-dimensional scaling (NMDS) analysis of bacterial communities associated with wheat, resulted in a 2-dimensional solution with a final stress of 16.4. However, NMDS analysis of bacterial communities associated with canola resulted in a 3-dimensional solution with a final stress of 11.5. Bacterial communities associated with root, stem and leaf of wheat and canola were grouped together, whereas rhizosphere communities were separated in different clusters (Figs. 4.1 and 4.2). In addition, shifts in bacterial profiles were detected among plant growth stages in wheat and canola. For example, bacterial communities associated with the studied crops exhibited similar DGGE profiles at stem elongation and flowering stages; however, a change in community structure was observed at plant ripening. In contrast, soils influenced bacterial profiles to a lesser extent.

4.5.2. Community Structure and Taxonomic Analyses of Bacterial Communities in the Plant Compartments based on High-Throughput Sequencing

The analysis of the bacterial communities, based on the high-throughput sequencing of 16S rRNA genes associated with canola and wheat, yielded 3,769,710 high quality sequencing reads, which corresponded to 9,857 OTUs. The number of sequences and OTUs was higher in canola compared to wheat (Table 4.2). In addition, rhizo-compartments (rhizosphere and root) in both crops exhibited the highest number of sequences and OTUs, followed by stems and leaves with seeds having the lowest values (Table 4.2). Venn diagram representing OTU distribution of each crop revealed that the rhizosphere, the root interior and the aboveground plant organs shared 359 and 449 OTUs in wheat and canola, respectively (Fig. 4.3). In both crops, the number of OTUs shared between the rhizosphere and aboveground plant organs was higher than those OTUs shared

between the root interior and aboveground plant organs (Fig. 4.3). In addition, the number of OTUs shared between the rhizosphere and root interior was higher in comparison with the number of OTUs shared among other plant compartments.

In wheat, the Chao 1 estimator indicated that bacterial richness was influenced ($P<0.05$) by the interaction soil × compartment, whereas the interaction soil × compartment × stage was significant ($P<0.05$) in canola (Table 4.3). The highest richness in both crops was detected in the rhizosphere, followed by the root, stem, leaf and seed (Table 4.4). In addition, the rhizosphere of both crops exhibited a higher bacterial richness in plants grown in the Brown Chernozem soil when compared to Black Chernozem soil. However, in other plant compartments, no significant differences in bacterial richness were observed amongst plants grown in different soils. Furthermore, the influence of growth stages on bacterial richness was only detected in the rhizosphere of canola in which a higher richness was observed at ripening when compared to stem elongation and flowering stages (Table 4.4).

Simpson index (1/D) indicated that bacterial diversity in wheat was influenced ($P<0.001$) by the interaction soil × compartment × stage, whereas the interaction soil × compartment was significant ($P<0.001$) in canola (Table 4.3). In both crops, the highest diversity was detected in the plant's rhizosphere whereas the lowest values were observed in the leaves (Tables 4.4). Similar to Chao1 estimator, the values of Simpson index observed in the rhizosphere of both crops were higher in the plants grown in Brown Chernozem soil compared to those grown in Black Chernozem soil, however, for other plant compartments no significant differences were observed for either soil. Additionally, Simpson index indicated that bacterial diversity did not vary significantly amongst the various growth stages in any of the compartments of canola. In contrast, the rhizosphere of wheat exhibited a higher diversity at the ripening stage, followed by flowering and stem elongation stages. However, in the root, stem, leaf and seed of wheat, no significant differences were detected among plant growth stages.

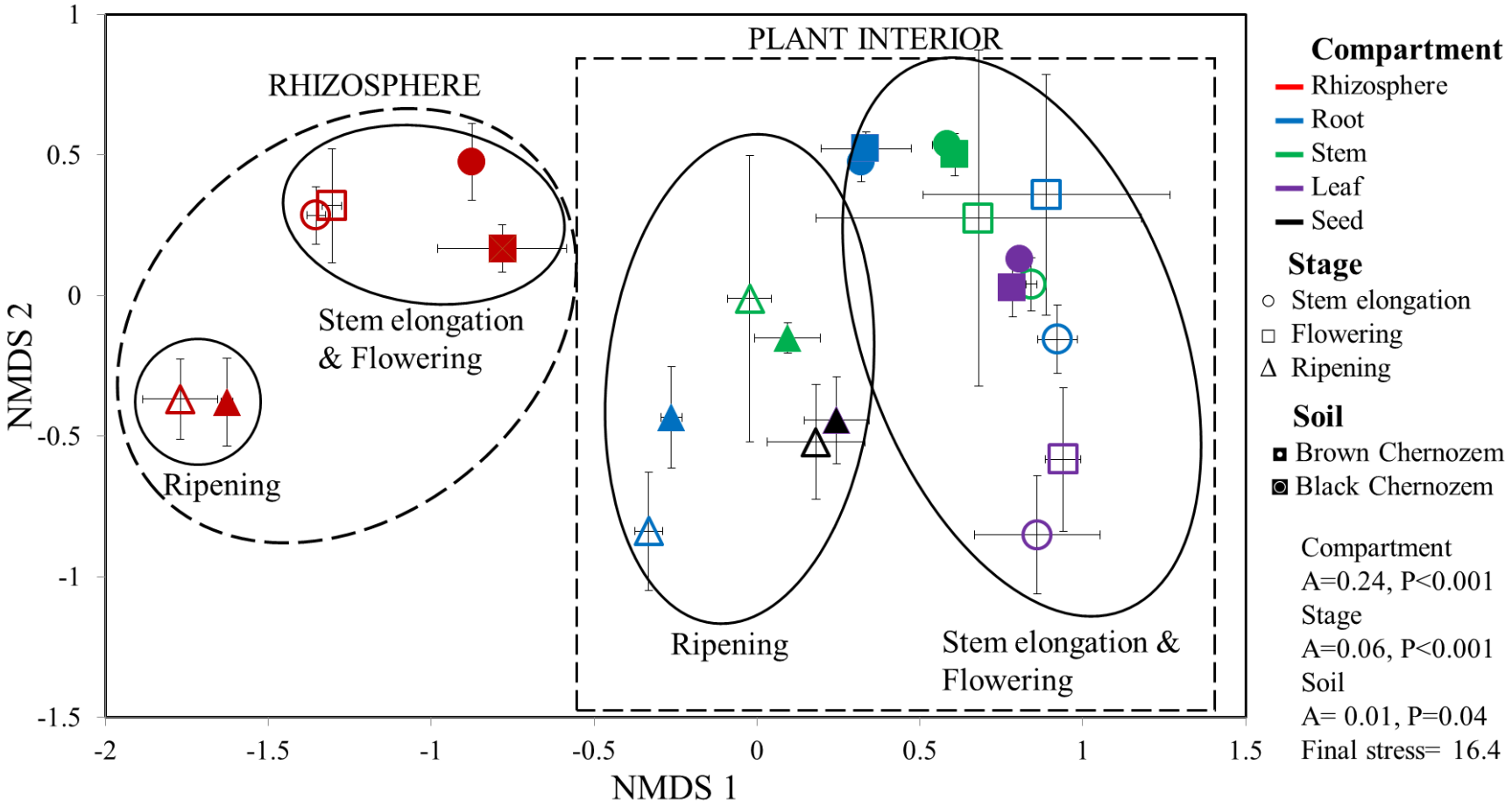

Fig. 4.1. Non-metric multidimensional scaling (NMDS) analysis for DGGE banding patterns of rhizosphere and plant associated bacterial 16S rRNA communities in wheat.

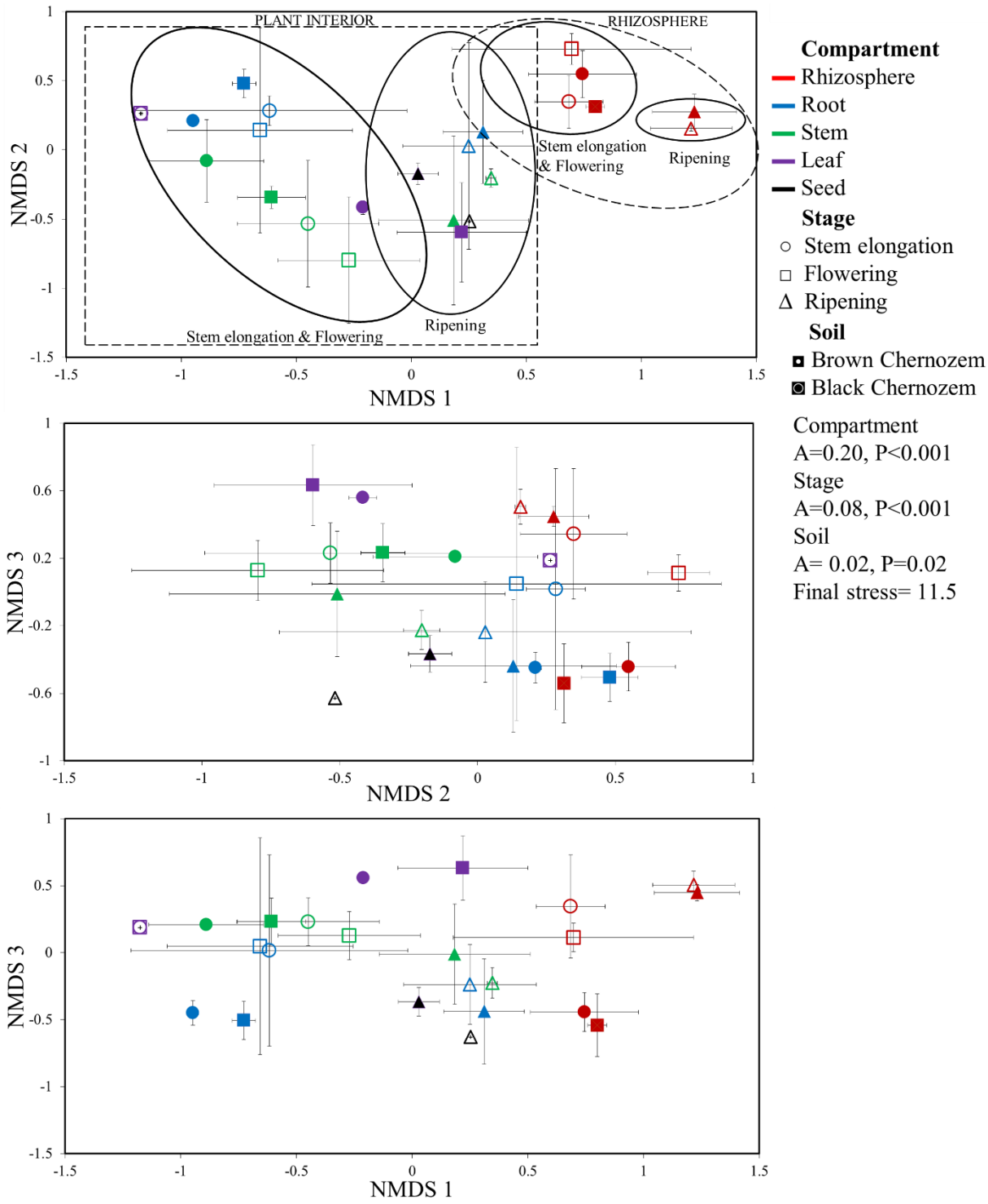

Fig. 4.2. Non-metric multidimensional scaling (NMDS) analysis for DGGE banding patterns of rhizosphere and plant associated bacterial 16S rRNA communities in canola.

Table 4.2. Number of bacterial sequences and OTUs in wheat and canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

Crop	Soil	Compartment	Sequences	OTUs
wheat	Brown	rhizosphere	474,997	5,906
		root	190,596	533
		stem	22,664	298
		leaf	24,307	182
		seed	4,966	114
	Black	rhizosphere	429,340	5032
		root	285,028	819
		stem	13,322	291
		leaf	4,521	234
		seed	482	101
	Total		1,450,223	8,453
canola	Brown	rhizosphere	480,725	6,096
		root	610,845	1,431
		stem	34,383	320
		leaf	65,132	172
		seed	1,754	103
	Black	rhizosphere	461,826	5,107
		root	549,861	1,328
		stem	73,477	470
		leaf	39,125	161
		seed	2,359	85
	Total		2,319,487	8,984
Grand Total			3,769,710	9,857

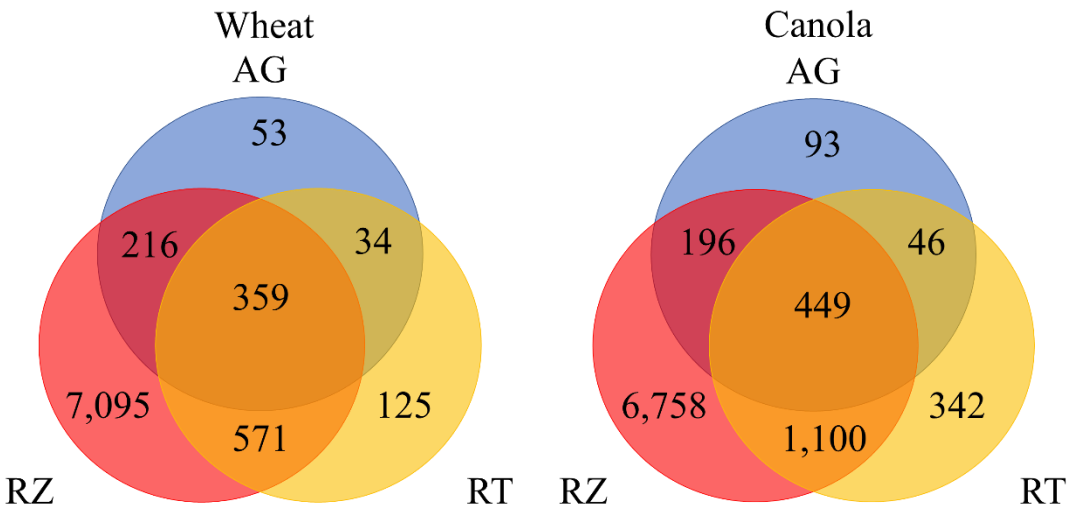

Fig. 4.3. Venn diagram representing the bacterial OTUs associated with the rhizosphere (RZ), root (RT) and aboveground plant organs (AG) of wheat and canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively. Data presented was obtained from plants harvested at stem elongation, flowering and ripening.

Table 4.3. ANOVA of richness (Chao 1) and diversity (1/D) of bacterial communities associated with wheat and canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

Source of variation	Wheat		Canola	
	Chao 1	1/D	Chao 1	1/D
Compartment	<0.0001 ***	<0.0001 ***	<0.0001 ***	<0.0001 ***
Soil	<0.0001 ***	<0.0001 ***	<0.0001 ***	<0.0001 ***
Stage	0.8 n.s.	0.1 n.s.	0.2 n.s.	0.6 n.s.
Compartment × Soil	<0.0001 ***	<0.0001 ***	<0.0001 ***	<0.0001 ***
Compartment × Stage	0.8 n.s.	<0.0001 ***	0.4 n.s.	0.1 n.s.
Soil × Stage	0.4 n.s.	<0.0001***	0.8 n.s.	0.4 n.s.
Compartment × Soil × Stage	0.7 n.s.	<0.0001 ***	0.04 *	0.9 n.s.

Note: *, **, ***, significant at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., not significant.

Table 4.4. Richness and diversity indexes of bacteria communities associated with wheat and canola grown in Brown (BR) and Black (BL) agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

			Wheat				Canola			
Soil	Compartment	Stage	Chao1		1/D		Chao1		1/D	
BR	Rhizosphere	Stem elongation	2895	n.s.	130.0	c	2856	a	129.6	n.s.
		Flowering	2896	n.s.	145.1	b	2978	a	142.2	n.s.
		Ripening	2923	n.s.	201.2	a	2987	a	135.6	n.s.
		Average	2905	a	158.7	a	2940	a	135.8	a
	Root	Stem elongation	142	n.s.	9.2	g	454	c	8.1	n.s.
		Flowering	117	n.s.	13.8	fg	375	cde	9.1	n.s.
		Ripening	207	n.s.	7.4	gh	426	cd	4.7	n.s.
		Average	155	cd	10.1	cd	419	c	7.3	cd
	Stem	Stem elongation	97	n.s.	19.0	fg	144	de	16.7	n.s.
		Flowering	161	n.s.	3.3	h	121	e	7.2	n.s.
		Ripening	73	n.s.	7.6	gh	82	e	5.1	n.s.
		Average	110	cd	10.0	cd	116	d	9.7	cd
	Leaf	Stem elongation	116	n.s.	5.6	gh	355	cde	3.2	n.s.
		Flowering	130	n.s.	1.1	h	111	e	1.9	n.s.
		Average	123	cd	3.4	d	233	cd	2.5	d
	Seed	Ripening	274	cd	22.9	f	140	de	5.7	cd
BL	Rhizosphere	Stem elongation	2448	n.s.	103.9	de	2459	b	93.7	n.s.
		Flowering	2338	n.s.	113.9	d	2291	b	93.0	n.s.
		Ripening	2374	n.s.	97.7	e	2393	b	97.4	n.s.
		Average	2386	b	105.1	b	2381	b	94.7	b
	Root	Stem elongation	300	n.s.	24.4	ef	445	c	8.1	n.s.
		Flowering	253	n.s.	14.5	fg	458	c	7.6	n.s.
		Ripening	187	n.s.	7.3	gh	356	cde	6.8	n.s.
		Average	247	c	15.4	c	420	c	7.5	cd
	Stem	Stem elongation	116	n.s.	5.4	gh	121	e	18.9	n.s.
		Flowering	108	n.s.	4.5	h	134	de	4.7	n.s.
		Ripening	78	n.s.	8.2	gh	117	e	9.1	n.s.
		Average	101	d	6.0	d	124	cd	10.9	c
	Leaf	Stem elongation	114	n.s.	5.1	gh	140	de	2.7	n.s.
		Flowering	161	n.s.	3.5	h	119	e	1.7	n.s.
		Average	137	cd	4.3	d	130	d	2.2	d
	Seed	Ripening	174	cd	3.2	h	85	e	2.7	cd

Note: Different letters indicate significant differences at $P \leq 0.05$ using Tukey's post hoc test. n.s., not significant.

Community structure of bacterial communities associated with the rhizosphere and root were analyzed separately from the aboveground plant organs (*i.e.*, stem, leaf and seed). Principal coordinate analysis (PCoA) of rhizosphere and root bacterial communities resulted in a 3-dimensional solution in which, PC1, PC2 and PC3 accounted for 33%, 12% and 9% of the variation of the bacterial profiles, respectively (Fig. 4.4). Results indicated that rhizosphere and root endophytic communities were clustered according to soil and/or crop species. Rhizosphere bacterial communities and root endophytes exhibited two clusters each. The first corresponded to Black and Brown Chernozem soils, whereas the latter corresponded to canola and wheat plants. Although clustering was observed based on plant species and soils, no clusters were observed amongst plant growth stages. The PCoA analysis of stem, leaf and seed bacterial communities also resulted in a 3-dimensional solution in which, PC1, PC2 and PC3 accounted for 19%, 6% and 5% of the variation of the bacterial profiles, respectively (Fig. 4.5). In the current study, endophytic bacterial communities associated to aboveground plant organs of wheat and canola exhibited no clustering among samples in response to soils, crop species or plant growth stages.

Taxonomic classification of the bacterial OTUs from potted-soil grown wheat and canola revealed 15 phyla. Overall, 11 bacterial phyla were observed concomitantly in canola and wheat grown in Brown and Black Chernozem soils (Figs. 4.6 and 4.7). Proteobacteria, Actinobacteria, Gemmatimonadetes, Bacteroidetes and Firmicutes were the dominant phyla in both wheat and canola plants. The phylum Proteobacteria was identified as the dominant bacterial taxa in both crops. Proteobacteria exhibited a higher relative abundance on the leaf and seed compartment when compared to rhizosphere, root and/or stem. In contrast, the relative abundance of the phylum Gemmatimonadetes was higher in the rhizosphere (Figs. 4.6 and 4.7). Additionally, candidate phylum SR1 was only observed in canola grown in Brown Chernozem soil (Figs. 4.6 B and 4.7 A). The phylum Chloroflexi could not be detected in wheat and/or canola grown in Brown Chernozem soil (Fig. 4.6 A), whereas Tenericutes could not be detected in plants grown Black Chernozem soils (Fig. 4.6 B).

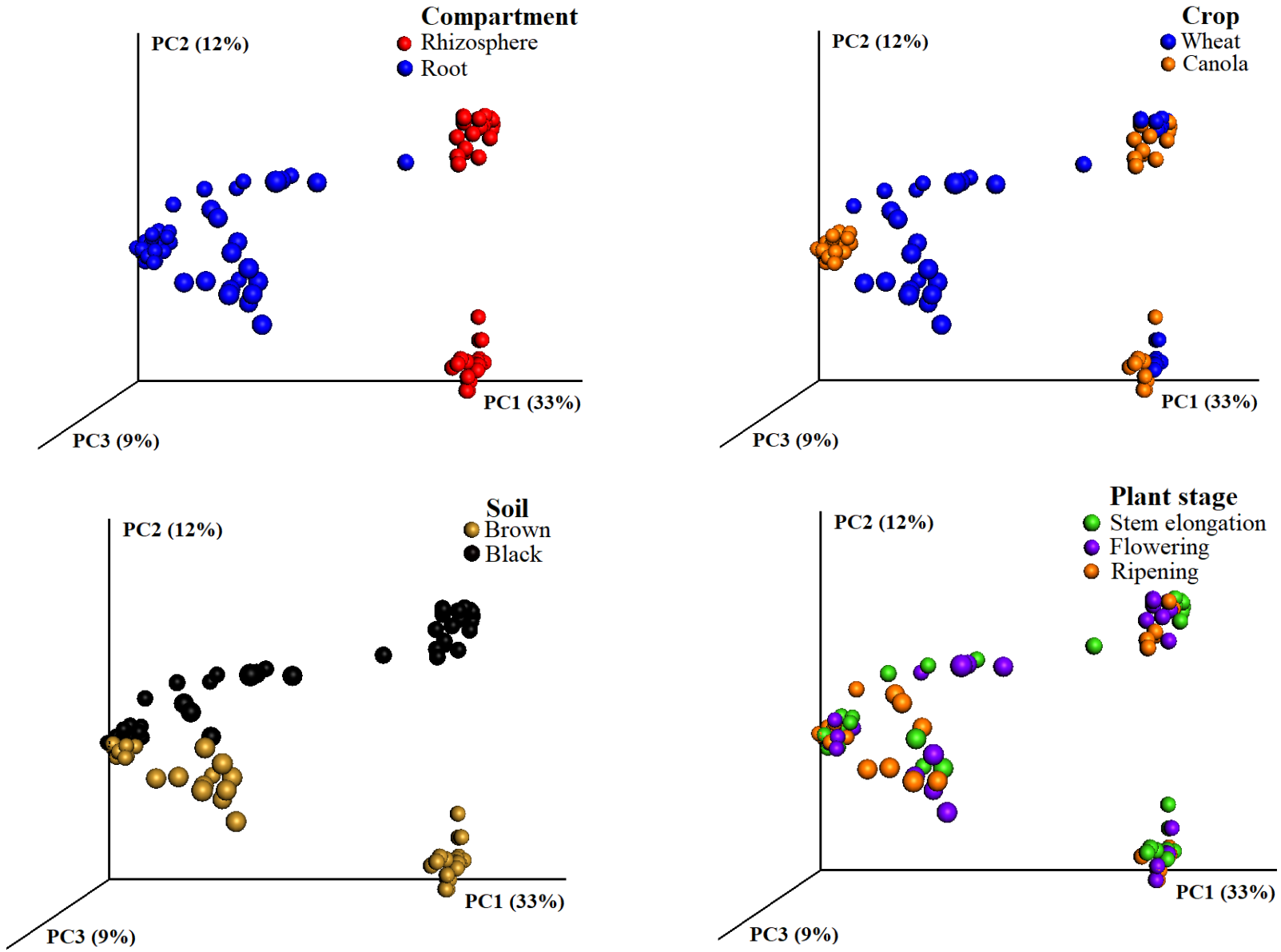

Fig. 4.4. Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity between rhizosphere and root bacterial communities associated to wheat and canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

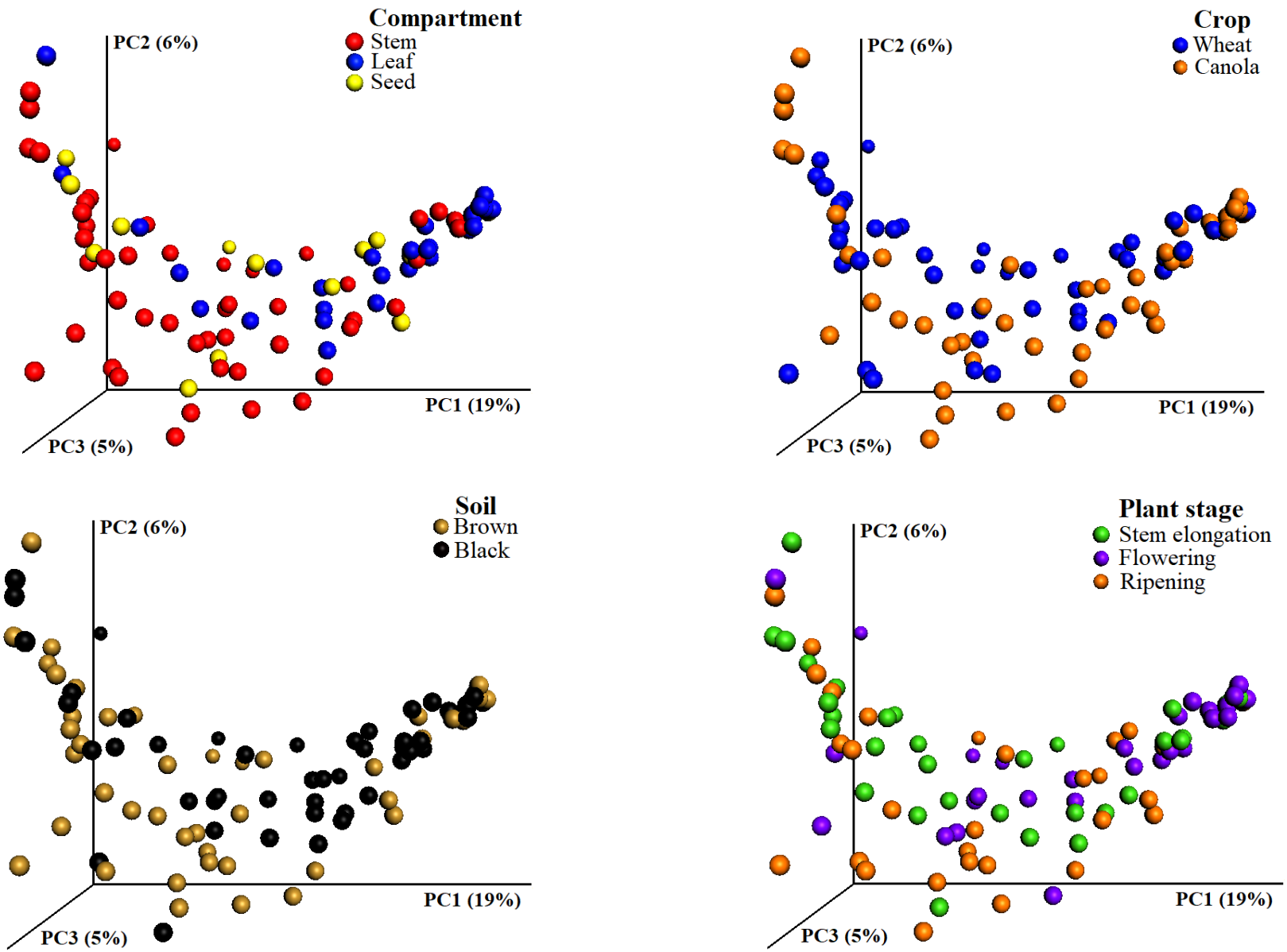

Fig. 4.5. Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity between stem, leaf and seed bacterial communities associated to wheat and canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

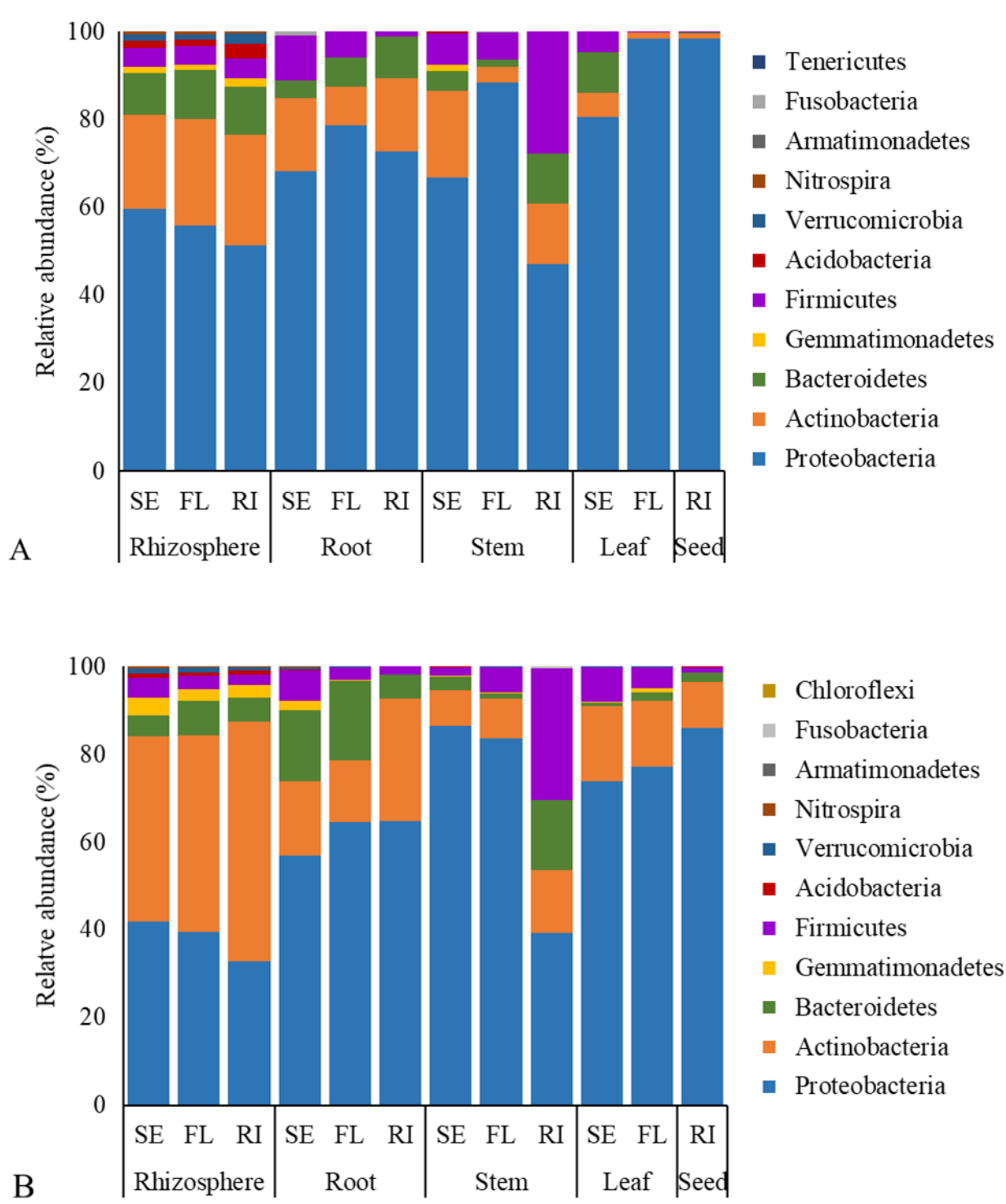

Fig. 4.6. Relative abundance of bacterial phyla associated with wheat grown in a Brown (A) and Black (B) agricultural soils from Central Butte and Melfort, Saskatchewan, respectively. Plants were harvested at Stem Elongation (SI), Flowering (FL) and Ripening (RI).

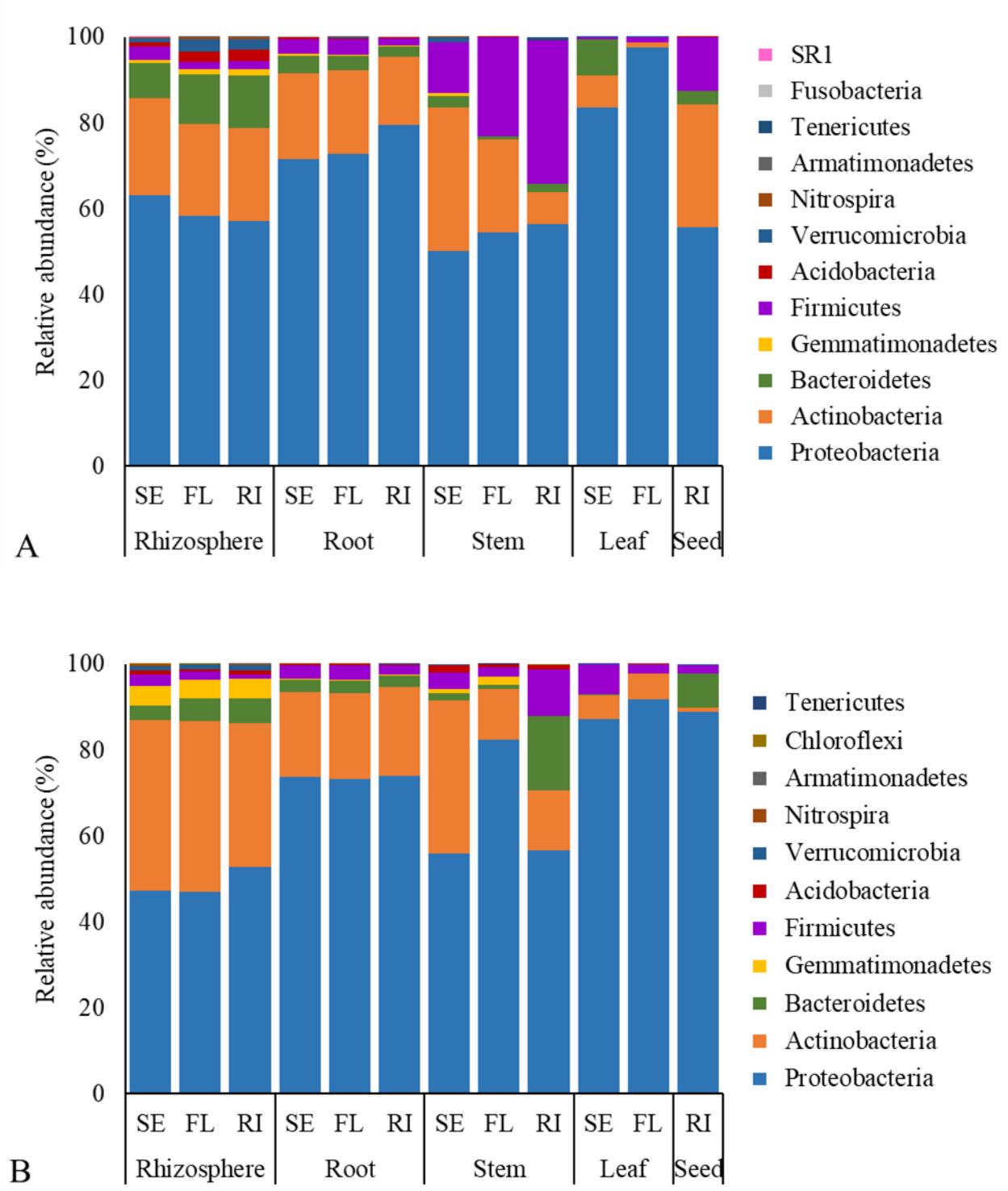

Fig. 4.7. Relative abundance of bacterial phyla associated with canola grown a Brown (A) and Black (B) agricultural soils from Central Butte and Melfort, Saskatchewan, respectively. Plants were harvested at Stem Elongation (SI), Flowering (FL) and Ripening (RI).

The most abundant bacterial genera associated with the rhizosphere and root interior of wheat were grouped into four main clusters: rhizosphere of wheat grown in Brown Chernozem soil (A) and in Black Chernozem soil (B), and root interior of wheat grown in Black Chernozem soil (C) and in Brown Chernozem soil (D) (Fig. 4.8). The most prevalent genera in the rhizosphere of wheat grown in Brown Chernozem soil (Cluster A) were *Lysobacter* (3%), *Sphingomonas* (3%) and unclassified genera of the families Burkholderiales (4%), Comamonadaceae (4%), Chitinophagaceae (4%), Rhizobiales (4%) and Sphingomonadaceae (3%). In addition, the rhizosphere of wheat grown in Black Chernozem soil (Cluster B) was dominated by *Arthrobacter* (4%), *Gemmatimonas* (3%), *Nocardiodes* (4%), *Solirubrobacter* (5%) and unclassified genera of the families Actinomycetales (3%), Geodermatophilaceae (4%), Rhizobiales (5%) and Solirubrobacterales (7%). In contrast, the root interior of wheat grown in Black Chernozem soil (Cluster C) exhibited a high abundance of *Chitinofaga* (7%), *Lysobacter* (4%), *Streptomyces* (10%), *Variovorax* (4%) and unclassified genera of the families Enterobacteriaceae (33%) and Oxalobacteraceae (4%). However, the bacterial communities associated the root interior of wheat grown in Brown Chernozem soil (Cluster D) mainly consisted of *Variovorax* (10%), *Devosia* (3%) and unclassified genera of the families Comamonadaceae (3%) Enterobacteriaceae (3%) and Pseudonocardiaceae (6%).

The most abundant bacterial genera associated with the rhizosphere and root interior of canola were grouped in three main clusters: root interior of canola grown in Brown and Black Chernozem soils (A), rhizosphere of canola grown in Brown Chernozem soil (B) and in Black Chernozem soil (C) (Fig. 4.9). Root samples (Cluster A) were characterized by the prevalence of the genera *Streptomyces* (3%), *Variovorax* (3%), and unclassified genera of the families Enterobacteriaceae (38%) and Pseudonocardiaceae (11%). Additionally, the most dominant genera in the rhizosphere of canola grown in Brown Chernozem soil (Cluster B) were *Sphingomonas* (3%) and unclassified genera of the families Burkholderiales (7%), Comamonadaceae (4%), Chitinophagaceae (4%), Enterobacteriaceae (3%) Rhizobiales (3%) and Sphingomonadaceae (3%). In contrast, rhizosphere communities associated to canola grown in Black Chernozem soil (Cluster C) were mostly dominated by *Gemmatimonas* (5%), *Solirubrobacter* (4%), *Lysobacter* (4%), *Variovorax* (4%), *Sphingomonas* (3%), *Nocardiodes* (3%), and unclassified genera of the families Geodermatophilaceae (3%), Rhizobiales (3%), Solirubrobacterales (5%) and Xanthomonadaceae (5%).

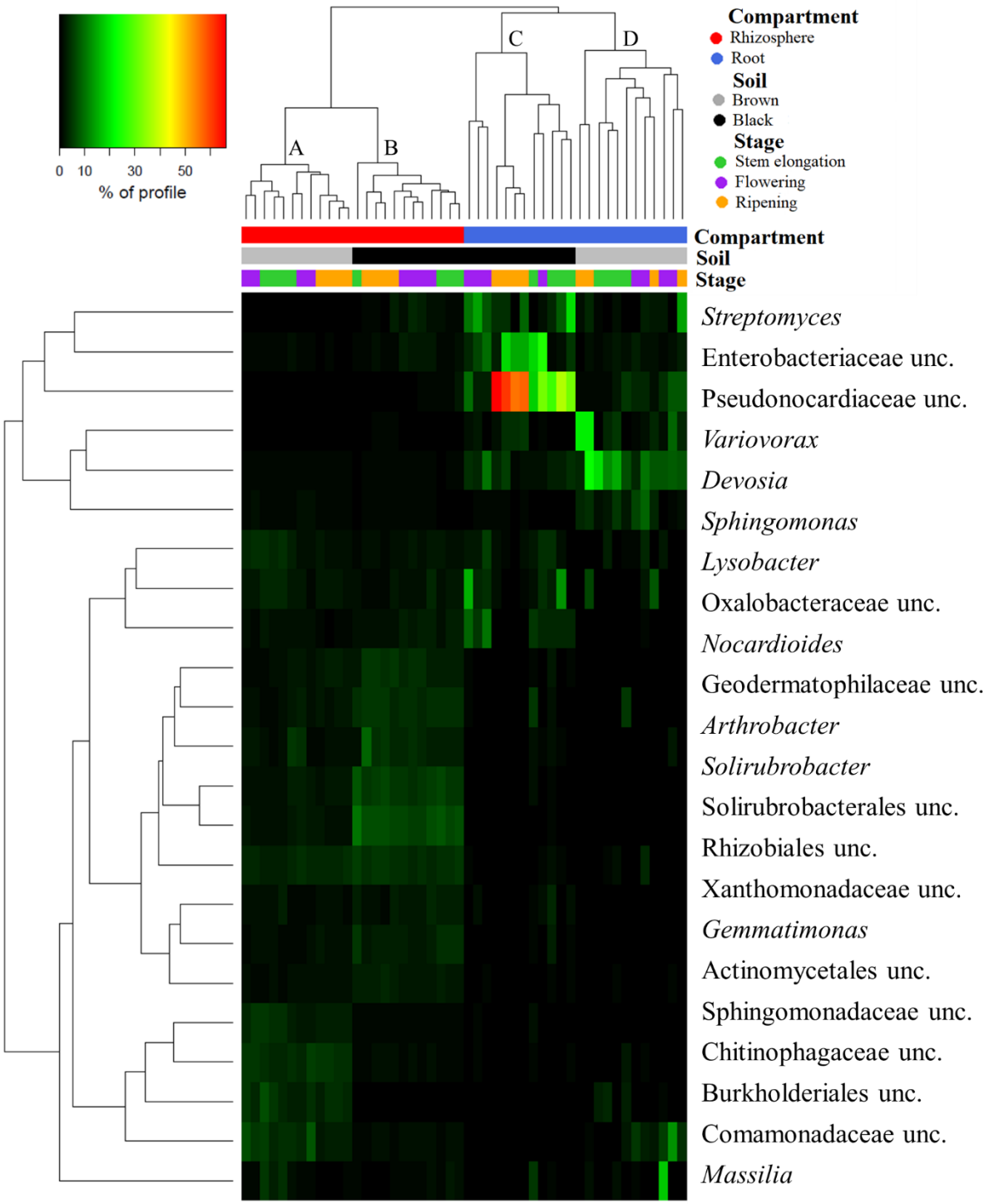

Fig. 4.8. Hierarchical clustering (Bray-Curtis) of bacterial genera (>1% abundant) associated with the rhizosphere and root of wheat grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

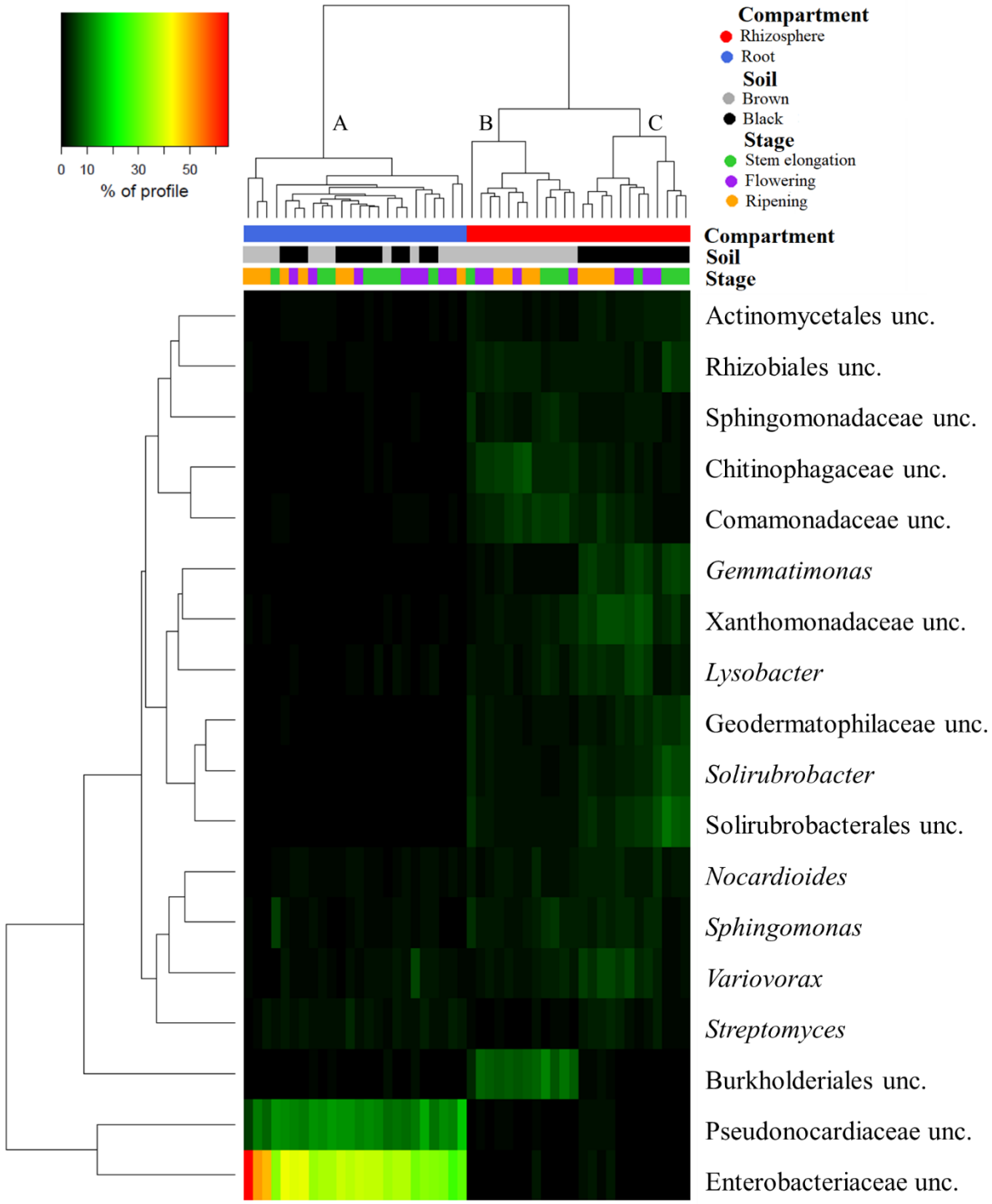

Fig. 4.9. Hierarchical clustering (Bray-Curtis) of bacterial genera (>1% abundant) associated with the rhizosphere and root of canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

The relative abundance of bacterial genera associated with the aboveground plant organs of wheat and canola was lower when compared to the rhizosphere and root samples (Fig. 4.10 and 4.11). The distribution of the bacterial genera associated with the aboveground plant organs in both crops was highly variable among soils and growth stages, thus no clusters were detected in response to those factors. Overall, the relative abundance of unclassified genera of the family Enterobacteriacaeae was high in the aboveground plant samples, accounting for 14-99% and 2-97% of bacterial communities associated to aboveground plant of wheat and canola, respectively (Figs. 4.10 and 4.11). Similarly, wheat and canola aboveground plant organs also exhibited a high abundance of *Corynebacterium* and *Pseudomonas* that accounted for up to 40% and 37% of the bacterial profile, respectively. In addition, in wheat aboveground plant organs also included *Tumebacillus* (2%) and unclassified genera of the family Planococcaceae (2%). Conversely, *Acinetobacter* (2%), *Staphylococcus* (2%) and unclassified genera of the order Actinomycetales (2%) were also detected in canola plants (Figs. 4.10 and 4.11). Furthermore, in both canola and wheat additional genera including mainly members of the phyla Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes were only detected in the aboveground plant organs and the relative abundance ranging from 0.2% to 0.001% of the bacterial profile of the stem, leaf and/or seed (Tables 4.5).

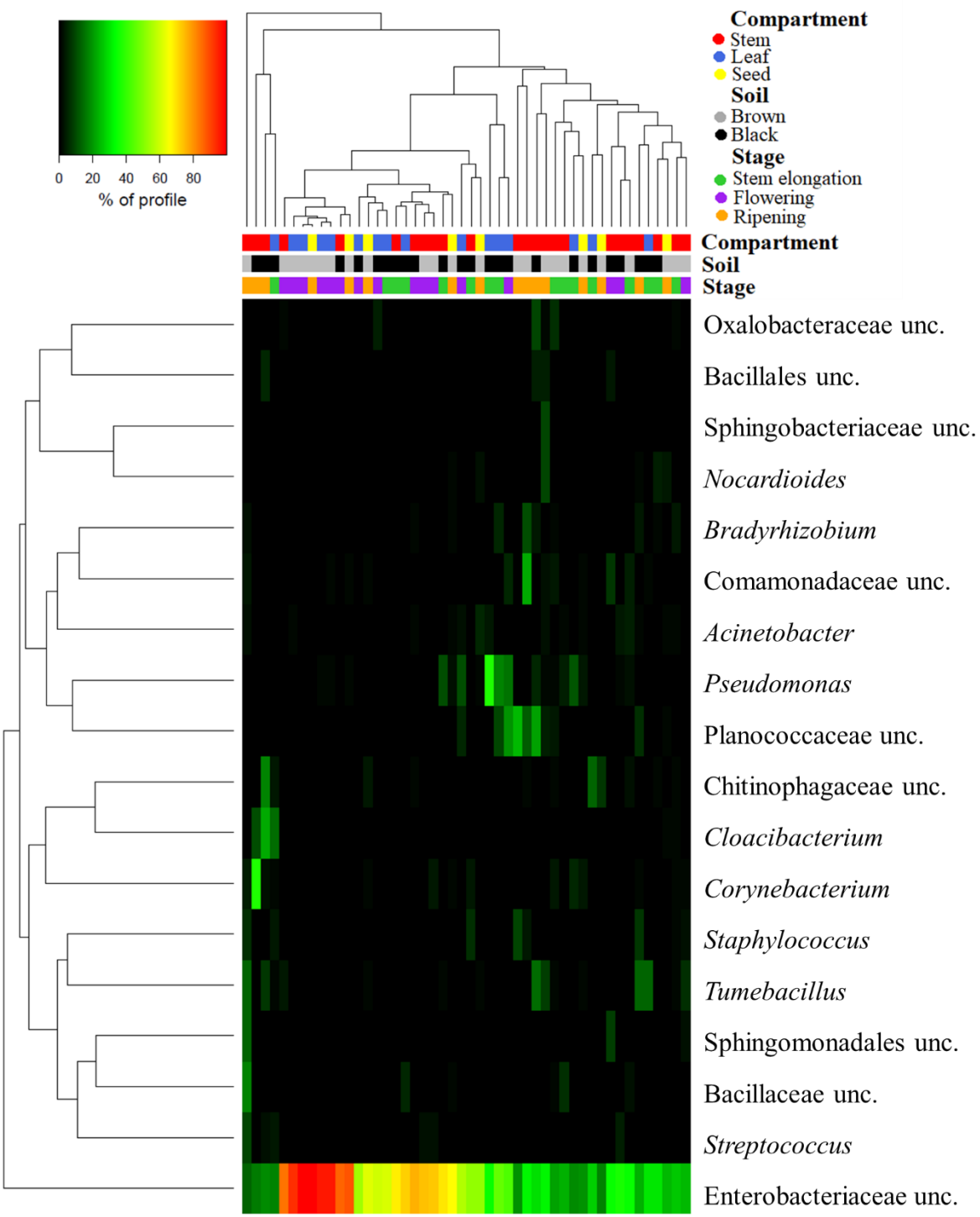

Fig. 4.10. Hierarchical clustering (Bray-Curtis) of bacterial endophytes genera (>0.5% abundant) associated with stem, leaf and seed of wheat grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

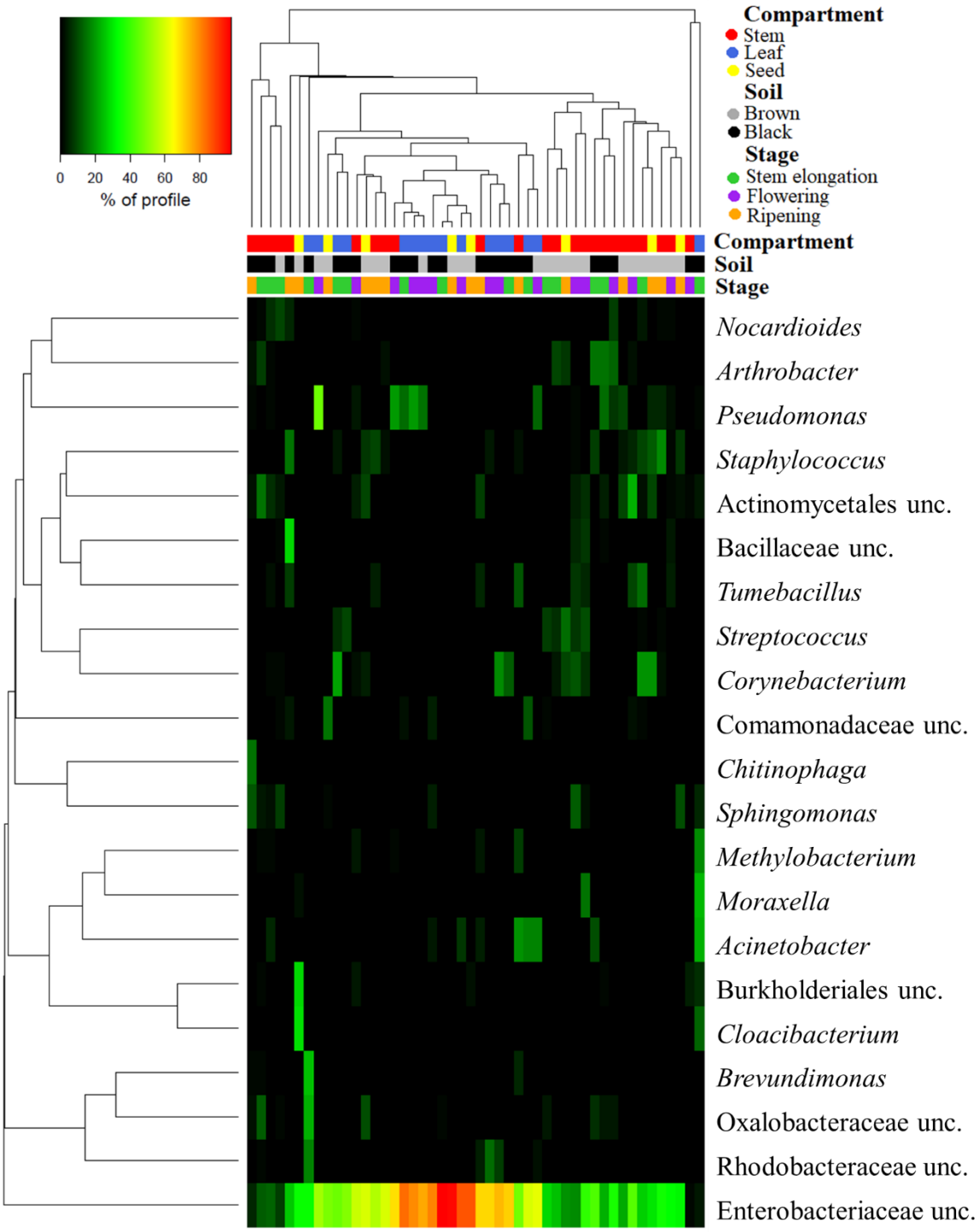

Fig. 4.11. Hierarchical clustering (Bray-Curtis) of bacterial endophytes genera (>0.5% abundant) associated with stem, leaf and seed of canola grown in Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

Table 4.5. Relative abundance (%) of endophytic bacteria only detected in the stem, leaf and seed of wheat and canola grown in agricultural soils from Saskatchewan.

Wheat				
		Stem	Leaf	Seed
		Relative abundance (%)		
Genus	Phylum			
Actinomyces	Actinobacteria	0.011	0	0
Corynebacterium	Actinobacteria	0.051	0.007	0.001
Gordonia	Actinobacteria	0.200	0.017	0
Kocuria	Actinobacteria	0.064	0.047	0
Segniliparus	Actinobacteria	0.003	0.714	0
Smaragdicoccus	Actinobacteria	0.008	0	0
Bacteroides	Bacteroidetes	0.014	0	0
Ohtaekwangia	Bacteroidetes	0.003	0	0
Paludibacter	Bacteroidetes	0.008	0	0
Prevotella	Bacteroidetes	0.013	0	0
Wautersiella	Bacteroidetes	0.003	0.100	0
Finegoldia	Bacteroidetes	0.086	0	0.003
Pediococcus	Bacteroidetes	0.189	0	0
Tumebacillus	Bacteroidetes	0	0.017	0
Fusobacterium	Fusobacteria	0.039	0	0
Alkanindiges	Proteobacteria	0.047	0	0
Enhydrobacter	Proteobacteria	0	0	0.006
Neisseria	Proteobacteria	0.028	0	0
Pedomicrobium	Proteobacteria	0.308	0	0
Psychrobacter	Proteobacteria	0.072	0	0

Canola				
		Stem	Leaf	Seed
		Relative abundance (%)		
Genus	Phylum			
Actinomyces	Actinobacteria	0.000	0.027	0
Bifidobacterium	Actinobacteria	0.031	0	0
Corynebacterium	Actinobacteria	0	0	0.170
Demequina	Actinobacteria	0.017	0	0
Rhodococcus	Actinobacteria	0	0	1.118
Bacteroides	Bacteroidetes	0	0	0.316
Chryseobacterium	Bacteroidetes	0.169	0	0
Hymenobacter	Bacteroidetes	0.032	0	0
Ohtaekwangia	Bacteroidetes	0.333	0	0
Wautersiella	Bacteroidetes	0.087	0	0
Aerococcus	Firmicutes	0.014	0	0
Anaerostipes	Firmicutes	0.088	0	0
Clostridia	Firmicutes	0.025	0	0
Faecalibacterium	Firmicutes	0.019	0	0
Jeotgalicoccus	Firmicutes	0	0	0.243
Macrococcus	Firmicutes	0	0.229	0
Mogibacterium	Firmicutes	0.130	0	0
Thermicanus	Firmicutes	0.001	0	0
Acinetobacter	Proteobacteria	0.057	0	0
Halomonas	Proteobacteria	0.070	0.001	0
Naxibacter	Proteobacteria	0.038	0	0
Neisseria	Proteobacteria	0.006	0	0
Pseudomonas	Proteobacteria	0	0.001	0
Sphingomonas	Proteobacteria	0.817	0	0

4.5.3. Influence of Plant Development Stages on Bacterial Microbiome

Bacterial communities associated with the rhizosphere, roots and stems of canola and wheat grown in agricultural soils were analyzed for their bacterial profiles at different plant growth stages (Figs. 4.12 and 4.13). The leaves and seeds of both crops were excluded from this analysis due to their absence at some of the plant development stages analyzed. The predominance of the most abundant bacterial families at stem elongation, flowering and ripening was influenced by crop species and plant compartments. For example, the bacterial profile in the rhizosphere exhibited an even distribution on the relative abundance of most families among growth stages in both wheat and canola crops. However, the family Enterobacteriaceae was higher in the rhizosphere of canola at ripening when compared to stem elongation and flowering (Figs. 4.12 and 4.13).

Analysis of the relative abundance of bacteria families in the canola roots revealed that bacterial communities exhibited an even distribution among various plant growth stages (Fig. 4.13). In contrast, wheat roots exhibited a more variable bacterial distribution among plant growth stages. For example, at stem elongation stage, wheat roots were enriched with families Bacillaceae, Geodermatophilaceae, Gemmatimonadaceae, Planococcaceae and Solirubrobacteraceae, whereas the family Oxalobacteraceae was predominant at flowering stage. Additionally, at ripening a high abundance of the families Bradyrhizobiaceae, Comamonadaceae, Enterobacteriaceae, Hyphomicrobiaceae, Mycobacteriaceae, Pseudonocardiaceae, Rhizobiaceae and Streptomycetaceae was detected (Fig. 4.12).

When compared to the rhizosphere and roots, the predominance of bacterial families associated with wheat and canola stems varied greatly among plant growth stages. For example, in wheat stem at stem elongation stage, most of bacterial families were scarce or absent. In contrast, the families Enterobacteraceae, Geodermatophilaceae, Pseudonocardiaceae, Solirubrobacteraceae and Streptomycetaceae dominated wheat stems at flowering. At ripening, the families Alicyclobacillaceae, Bacillaceae, Bradyrhizobiaceae, Chitinophagaceae, Nocardioidaceae, Planococcaceae and Sphingobacteriaceae were also prevalent in wheat stem (Fig. 4.12). Conversely, in canola stems the relative abundance of most families was higher at stem elongation and flowering stages, except that families Chitinophagaceae and Sphingobacteriaceae were highly prevalent at ripening (Fig. 4.13).

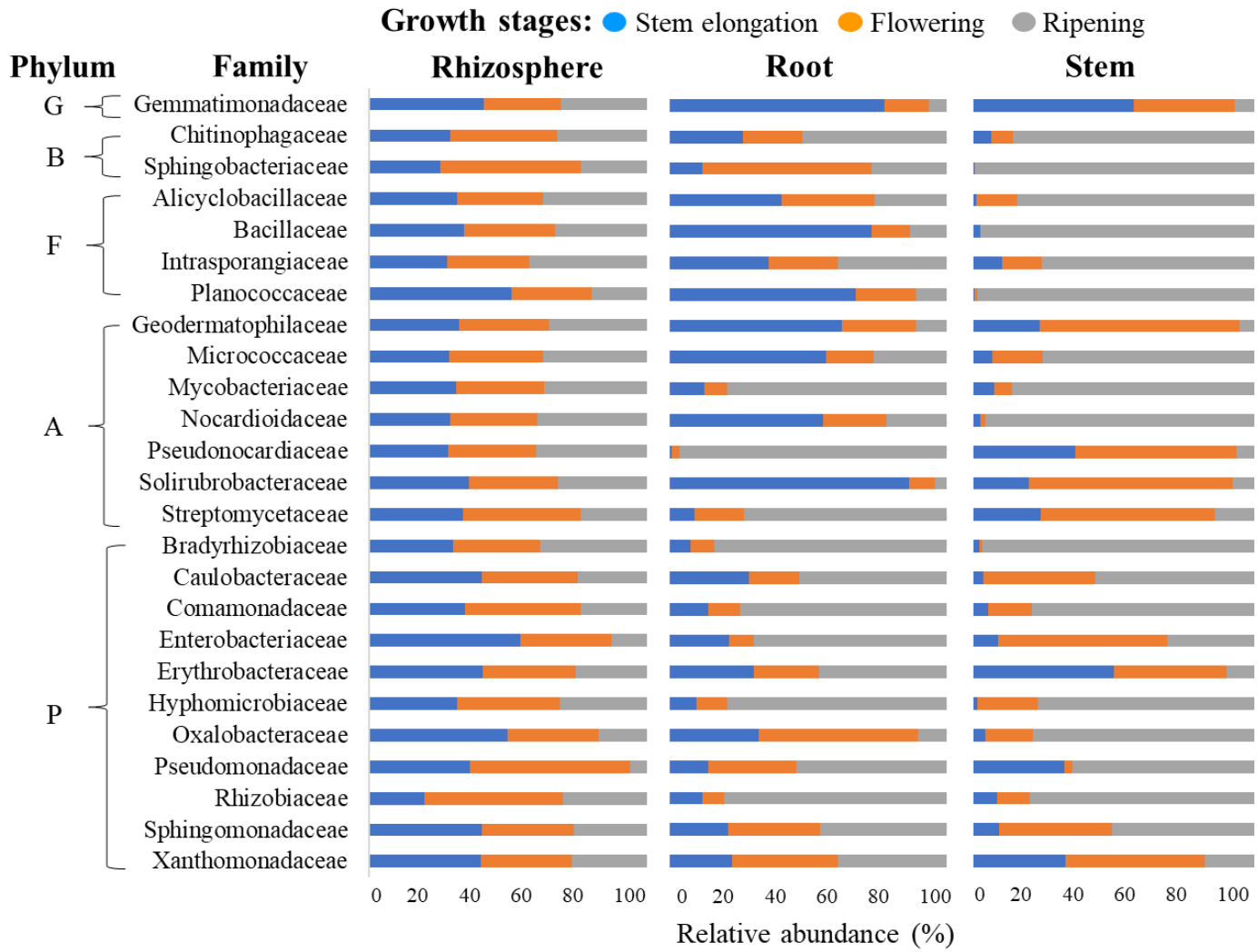

Fig. 4.12. Relative abundance of bacterial families associated with rhizosphere, root and stems of wheat grown in agricultural soils from Saskatchewan at stem elongation, flowering and ripening. Families were classified by phyla: Proteobacteria (P), Actinobacteria (A), Firmicutes (F), Bacteroidetes (B) and Gemmatimonadetes (G).

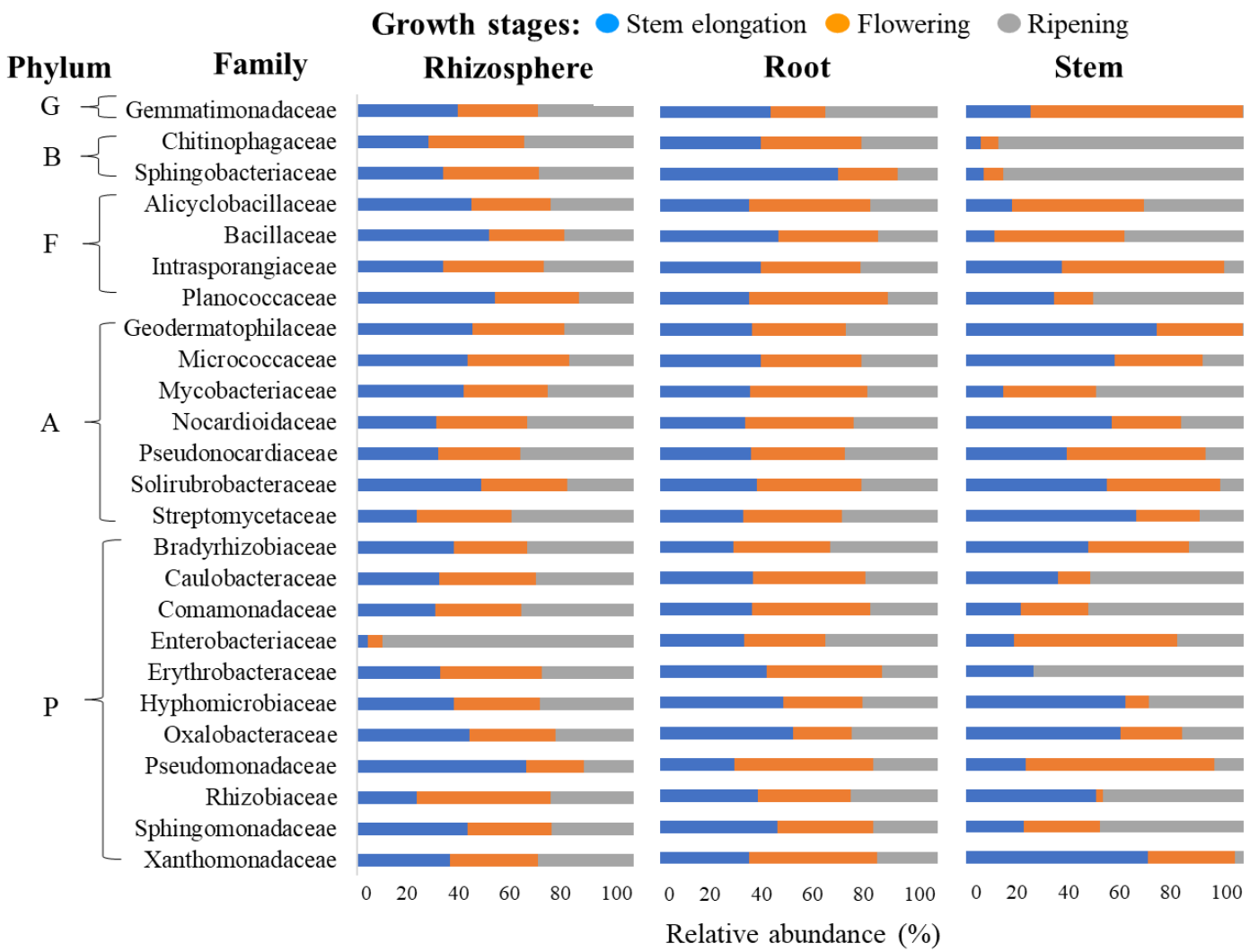

Fig. 4.13. Relative abundance of bacterial families associated with rhizosphere, root and stems of canola grown in agricultural soils from Saskatchewan at stem elongation, flowering and ripening. Families were classified by phyla: Proteobacteria (P), Actinobacteria (A), Firmicutes (F), Bacteroidetes (B) and Gemmatimonadetes (G).

4.6. Discussion

In this study, the diversity of bacterial communities associated with rhizosphere and plant interior of wheat and canola was investigated. High-throughput sequencing (Figs. 4.4 and 4.5) and DGGE (Figs. 4.1 and 4.2) analyses suggested that bacterial community structure of wheat and canola differed among the rhizosphere, root and aboveground plant organs. In addition, the rhizosphere and roots in both crops exhibited a higher number of sequences and OTUs compared to the aboveground plant organs (Table 4.2). These findings suggest that endophytic bacterial communities were a subset of the rhizosphere microbiome (Germida et al., 1998; Bulgarelli et al., 2013; Edwards et al., 2015). The literature proposes several possible mechanisms that may be involved in bacterial root colonization including: (*i*) the recognition of specific compounds in the root exudates by the bacteria, (*ii*) bacteria chemotaxis towards those compounds, (*iii*) bacterial penetration into the root, and (*iv*) bacteria multiplication in the intercellular spaces (Hardoim et al., 2008). In addition, bacteria may also translocate from roots to aboveground plant organs by accessing the interior of xylem vessels (Compant et al., 2010). These multiple steps required for the bacterial establishment within the plant tissues involve bacteria physiological traits such as the production of lipopolysaccharides, the activity of flagella and/or pili, the secretion of cell-wall degrading enzymes and the degradation of plant-derived compounds (Compant et al., 2010). In addition, the lower diversity and species richness of the bacterial communities associated with wheat and canola aboveground plant organs (Tables 4.2 to 4.4, Fig 4.3) may be related to the low number of endophytes that are able to colonize aerial plants organs. In fact, Hallmann (2001) reported that bacterial endophytes have to overcome several plant morphological barriers and physiological limitations to establish in the stem, leaves and seeds.

Analysis of bacterial community structure associated with wheat and canola indicated that rhizosphere communities were mainly influenced by soil characteristics (Fig. 4.4). Previous studies reported that soil properties have the ability to influence the bacterial communities, not only in the bulk soil, but also microbial communities in the plant's rhizosphere (Garbeva et al., 2004). In the current study, however, root bacterial endophytes differed between wheat and canola communities (Figs. 4.1, 4.2 and 4.4) suggesting that each crop selects distinct bacterial communities. Bulgarelli et al. (2013) reported that the release of root exudates in the rhizosphere is important role for the selection of root microbiota by the crop. The selection of endophytic communities from the rhizosphere occurs during the root colonization as the host crop regulates

the colonization and multiplication of specific bacterial taxa in the root interior. For example, several plant characteristics such as plant health, plant developmental stages, root morphology, composition of root exudates or the presence of plant wounds may affect the penetration of bacteria into the host plant roots (Garbeva et al., 2004, Gaiero et al., 2013). Additionally, the plant innate immune system may also influence the selection of a particular root endophyte microbiota. Boller and He (2009) suggested that some bacterial endophytes may possess effective mechanisms to avoid detection a plant's immune system and thus establish viable colonies inside the plant tissues. Conversely in the current study, crop species and/or soil characteristics did not influence the community structure of bacterial endophytes associated with either wheat or canola aboveground plant organs (Fig. 4.5). Previous studies have indicated that microbial communities associated with the aboveground plant organs are influenced by several abiotic factors such as temperature, humidity, light irradiation and access to nutrients, which are more fluctuating in the aboveground plant organs in comparison to the rhizosphere and root environments (Hirano and Upper, 2000). The high variability in the bacterial profiles detected in the stems, leaves and seeds of wheat and canola (Fig. 4.5) may be related to the diverse habitats colonized by bacterial in the aboveground plant organs (Compant et al., 2010). In addition, these bacterial endophytic communities may originate from different environments such as the phyllosphere (Frank et al., 2017).

In the current study, the phyla Proteobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes and Firmicutes were the dominant bacteria associated to the rhizosphere of wheat and canola (Figs. 4.6 and 4.7). Previous studies assessing the bacterial communities of wheat rhizosphere revealed an enrichment of Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria and Planctomycetes (Turner et al., 2013b). Additionally, Donn et al. (2015) also reported the presence of Actinobacteria, Bacteroidetes and Proteobacteria associated with wheat. Similar to this study, bacterial communities associated with the rhizosphere of canola plants cultivated on agricultural soils from Ottawa, Canada, also exhibited Proteobacteria, Actinobacteria and Gemmatimonadetes (Monreal et al., 2017). An additional related crop such as winter *Brassica napus* was also dominated by Actinobacteria, Bacteroidetes and Proteobacteria (Gkarmiri et al., 2017; Rathore et al., 2017). These results suggest a selection of certain bacterial phyla in the rhizosphere of the studied crops. Bulgarelli et al. (2013) concluded that the establishment of rhizosphere bacterial community is modulated by the secretion of photoassimilates from root cells. In fact, the high abundance of Proteobacteria and Bacteroidetes in the rhizosphere of various crops

is attributed to their fast-growing capacity and higher efficiency in metabolizing root exudates (Fierer et al., 2007; García-Salamanca et al., 2013; Peiffer et al., 2013). Furthermore, the phylum Actinobacteria is reported to have a high persistency in soils (Van Elsas et al., 2006), which may explain the high abundance of this phylum in the rhizosphere of the studied crops.

Similar to the rhizosphere bacteria profiles, in the current study the plant interior of wheat and canola was also enriched with Actinobacteria, Bacteroidetes and Proteobacteria (Figs. 4.6 and 4.7). However, the endophytic community colonizing plant organs had a lower prevalence of Gemmatimonadetes and Firmicutes, thus suggesting that crops select specific bacterial phyla during endophytic colonization of the roots and aboveground plant organs. A previous study by Rascovan et al. (2016) reported a high abundance Proteobacteria, Firmicutes, Planctomycetes and Verrucomicrobia in the root interior of wheat; although, Ofek et al. (2014) only detected Proteobacteria and Actinobacteria as the dominant phyla in wheat. Similarly, studies conducted by de Campos et al. (2013); Gkarmiri et al. (2017) and Rathore et al. (2017) demonstrated that the root interior of winter *Brassica napus* and canola also comprised of a high abundance of Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. Furthermore, previous studies assessing the stem and leaf microbiome associated with wheat also demonstrated that the most abundant phyla were Proteobacteria, Bacteroidetes and Firmicutes, whereas Proteobacteria and Actinobacteria were highly prevalent in canola (Copeland et al., 2015; Gdanetz and Trail, 2017). Additional authors also reported that seed microbiome in canola exhibited a predominance of phylum Proteobacteria (Barret et al., 2016; Rybakova et al., 2017). Hirano and Upper (2000) reported that fluctuations in environmental conditions and nutrient availability could limit bacteria ability to colonizing aboveground plant organs successfully. However, Proteobacteria are fast-growing microorganisms with high efficiency in metabolizing carbon substrates (Fierer et al., 2007; García-Salamanca et al., 2013; Peiffer et al., 2013), thus conferring beneficial attributes that may contribute to their success in colonizing aboveground plant organs.

Rhizosphere bacteria associated with wheat and canola were characterized by the dominance of bacterial genera that have been demonstrated to exhibit beneficial effects on these crops (Figs. 4.8 and 4.9). For example, when the rhizosphere of wheat and canola grown in both Brown and Black Chernozem soils was assessed for bacterial diversity, the genus *Gemmatimonas* was predominant (Figs. 4.8 and 4.9). In fact, this genus is reported previously to be widely distributed in agricultural systems (DeBruyn et al., 2011; Gkarmiri, et al, 2017). However, these authors also

reported that a high relative abundance of Gemmatimonadetes when plants were grown in soils with pH near to neutrality and somewhat similar to the pH of the Brown and Black Chernozem soils used in the current study. DeBruyn et al. (2011) reported that Gemmatimonadetes exhibited a high desiccation tolerance, which may explain their high dispersal rates, and hence, their wide distribution in soils. In the current study, the rhizosphere of canola and wheat grown in Brown soil exhibited a high abundance of the genera *Solirubrobacter* and *Nocardioides* (Figs. 4.8 and 4.9), which have been previously detected in diverse soil types including tropical forest, desert and farmlands as well as the rhizosphere soil associated with wheat, canola, maize and poppy plants (Topp et al., 2000; Piutti et al., 2003; Donn et al., 2015; Tuo et al., 2015; Singh et al., 2016; Wang et al., 2017; Monreal et al., 2018; Tin et al., 2018). The genera *Solirubrobacter* and *Nocardioides* are classified as Actinobacteria, and are known for having high persistence in soils, even under low nutrient availability (Van Elsas et al., 2006). Furthermore, results in the current study also indicated that the families Commamonadaceae, Chitinophagaceae and Sphingomonadaceae were prevalent in the rhizosphere of canola and wheat grown in Black Chernozem soil. Previous studies also detected the abovementioned families in the rhizosphere soil associated with canola and wheat plants (Croes et al., 2013; de Campos et al., 2013; Donn et al., 2015; Gkarmiri, et al, 2017). Additionally, de Campos et al. (2013), Gkarmiri et al. (2017) and Monreal et al. (2018) reported a high abundance of Xanthomonadaceae in the rhizosphere soil associated with canola plants, which was confirmed in the current study (Fig. 4.9). Strains of *Xanthomonas maltophilia* have been described as a phosphate-solubilizing rhizobacteria that stimulated canola plant height (De Freitas et al., 1997), whereas, *Xanthomonas campestris* produced the bacterial black rot in canola plants (Kharbanda et al., 2001). These traits may suggest that association of Xanthomonadaceae with canola vary among bacterial species. Similar to the current study, Germida and Siciliano (2001) and Germida et al. (1998) also reported that *Arthrobacter* was detected in the rhizosphere of wheat, thus suggesting that *Arthrobacter* is amongst the most prevalent genera associated with the rhizosphere of wheat grown in Saskatchewan agricultural soils. Beneficial strains of *Arthrobacter* sp. have been used as inoculants in wheat to alleviate adverse effects of soil salinity on plants grown in saline conditions (Upadhyay et al., 2012).

In the current study, when roots of wheat grown in Saskatchewan soils were examined for bacterial diversity, a high abundance of *Stenotrophomonas*, *Streptomyces*, *Variovorax* was detected (Fig 4.8). These genera also are reported previously as beneficial microorganisms

associated with wheat. Many species of *Stenotrophomonas* are reported to ameliorate abiotic stress, control disease and promote plant growth in wheat plants (Dal Bello et al., 2002; Salantur et al., 2006; Ryan et al., 2009; Majeed at al., 2015; Singh and Jha, 2017). Additional authors (Coombs and Franco, 2003; Conn and Franco, 2004) also conducted studies that isolated culturable endophytic Actinobacteria from wheat and concluded that *Streptomyces* were the main genus associated with wheat roots. Species of *Streptomyces* are reported to exhibit biocontrol activity against wheat diseases such as Fusarium Head Blight and Take-all disease (Coombs et al., 2004; Mouloud et al., 2015). An additional genus *i.e*., *Variovorax* also are reported to be associated with wheat grown in agricultural soils from Argentina, Canada, France and United States (Germida et al., 1998; Bertrand et al., 2001; Germida and Siciliano, 2001; Yin et al., 2013; Rascovan et al., 2016). Species of *Variovorax* may be important for the mineralization of carbon bound sulfur in wheat, thus contributing to the ability of host plant to grow in soils with low sulfur availability (Schmalenberger et al., 2008). In the current study, the actinobacteria *Lentzea* was high abundant in the root interior of both canola and wheat plants grown in agricultural soils (Figs. 4.8 and 4.9), however, *Lentzea* also has been detected previously in wheat (Conn and Franco, 2004). Additionally, in the current study the endophytic *Pantoea* also was dominant in canola roots (Fig 4.9), but other species also have been reported in the rhizosphere and root interior of canola grown in soils from Brazil and Belgium (Farina et al., 2012; Croes et al., 2013). Some species of *Pantoea* also are reported previously to control phytopathogens and enhance plant growth of canola (Bardin et al., 2003; Trifi et al., 2017). These results suggest that the endophytic bacteria identified in the current study may be tested for beneficial activity in wheat and canola in future research.

In contrast to the root bacterial communities, endophytic bacteria associated with stem, leaf and seed of wheat and canola grown in soils from Saskatchewan exhibited similar community profiles among crops (Figs. 4.5, 4.10 and 4.11). This observation suggests that bacteria communities associated with aboveground plant organs were less influenced by crop genotype in comparison to the root associated bacteria (Peiffer et al., 2013; Ofek et al., 2014; Gdanetz and Trail, 2017). Other authors also reported a predominance of Enterobacteriaceae in the aboveground plant organs of wheat and canola. For example, *Erwinia* is reported as the prevalent genus in canola leaf and wheat seed (Copeland et al., 2015; Huang et al., 2016), whereas *Pantoea* was the dominant genus in canola seed (Barret et al., 2016). Some species of *Erwinia* and *Pantoea* are reported as beneficial microorganisms associated with wheat and canola. For instance, *Pantoea agglomerans*

strains increased the grain yield and water status of wheat, whereas in canola, they produced indole-3-acetic acid, controlled damping-off disease and promoted plant growth (Amellal et al., 1998; Remus et al., 2000; Bardin et al., 2003; Sergeeva et al., 2007). Similarly, *Erwinia herbicola* strains have been demonstrated to control seedling blight and blackleg diseases in wheat and canola, respectively (Kempf et al., 1989; Chakraborty et al., 1994). In contrast, other bacterial species of *Erwinia* have been reported to produce diseases in wheat and canola. For instance, *Erwinia rhapontici* and *Erwinia carotovora* produced pink seed in wheat and bacterial soft rot in canola, respectively (Khardanba et al., 2001; Hsieh et al., 2010). Results shown in the current study revealed that *Pseudomonas* was also prevalent in the aboveground plant organs of wheat and canola (Figs. 4.10 and 4.11). Several pseudomonads are reported previously to produce beneficial effects in wheat and canola including disease control and plant growth promotion (Thomashow et al., 1990; Germida and Walley, 1996; Bertrand et al., 2001; Amein and Weiber, 2002). Furthermore, in both canola and wheat several bacterial genera were only detected in the aboveground plant organs suggesting that these bacteria originated initially in the phyllosphere of the plant and unlikely in the root and/or soil (Table 4.5). Thus, microorganisms found in the phyllosphere may originate from various sources including the atmosphere, rain, and/or plant pollinators (Frank et al., 2017).

As indicated by DGGE and high-throughput sequencing analyses, the bacterial microbiome associated with wheat and canola at stem elongation, flowering and ripening varied among plant growth stages (Figs. 4.1, 4.2, 4.12 and 4.13). Earlier studies examining the influence of growth stages on the bacterial communities associated with the rhizosphere, root and leaves of wheat and canola also reported a shift on the bacterial communities among growth stages in field conditions (Smalla et al., 2001; Dunfield and Germida, 2003; Farina et al., 2012; de Campos et al., 2013; Copeland et al, 2015; Gdanetz and Trial, 2017). Similar to the results reported by Smalla et al. (2001) and Donn et al. (2015), the diversity of rhizosphere bacteria associated with wheat and canola was higher at ripening and flowering stages, when compared to the stem elongation growth stage (Table 4.6). Gdanetz and Trial (2017) suggested that the increase in the diversity of rhizosphere bacteria at flowering and maturity could be explained by three possible factors including: (*i*) the bacterial succession through growth stages is a consequence of root surface increases and hence high availability of habitats and resources in the rhizosphere; (*ii*) the shift of bacterial communities is due to signaling between host plant and microorganisms that colonized

the rhizosphere at earlier stages; and (*iii*) the bacterial response to the availabily of complex metabolites released by mature plant roots. In addition, the analysis of the relative abundance of bacteria families associated with wheat and canola indicated that the influence of the growth stages on the bacterial microbiome was crop and organ specific. For example, the variation in the relative abundance of bacterial families among plant growth stages in wheat root and stem was higher when compared to canola (Figs. 4.12 and 4.13). These results suggest that each crop may select specific bacterial taxa at each plant growth stage and within the different plant compartments. In fact, previous studies have concluded that the presence of certain bacterial groups at specific growth stages was related to the different ecological strategies within rhizosphere and plant bacterial communities and their interaction with the host crop. At stem elongation stage for example, the tip of the young roots provide the highest amount of organic carbon that can be rapidly used by fast growing and efficient carbon degrading bacteria (r-strategist), which may be advantageous when colonizing young plants (Brimecombe et al., 2000). In contrast, at ripening, bacterial communities have been reported to be dominated by K-strategists *i.e.*, bacteria exhibiting low growth rates and high persistency, even under low nutrient availability (Chiarini et al., 1998; Brimecombe et al., 2000).

4.7. Conclusions

This study provides insights on the diversity of the bacterial microbiome associated with plant organs of wheat and canola grown in agricultural soils from Saskatchewan, Canada. Bacterial community structure in both crops exhibited remarkable differences between the rhizosphere, root and aboveground plant organs. Rhizosphere bacteria associated with the studied crops differed between plants grown in Brown and Black Chernozem soils, suggesting that soil properties influenced the rhizosphere microbiome. Crop species influenced endophytic bacteria associated with the roots of the crops. In contrast, the aboveground plant organs exhibited a high variability among crops and soils, thus suggesting that additional environmental factors may have influenced the bacterial microbiome in these plant organs. These results suggest that the possible transmission and dynamic of bacteria in the rhizosphere, root and aerial plant organs of crops may be controlled by distinctive factors such as soil properties, plant genotypes and environmental factors. Additionally, plant growth stages also may have influenced the bacterial microbiome associated with the rhizosphere, root and aboveground plant organs of wheat and canola and the effect of growth stages on bacterial communities was crop and organ specific.

5. GROWTH PROMOTING POTENTIAL OF BACTERIAL ENDOPHYTES ASSOCIATED WITH CROPS

5.1. Preface

The previous study (Chapter 3) revealed that canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown in agricultural fields from Saskatchewan, Canada, selected specific bacterial consortia within their roots. Bacterial endophytes strains were isolated from the roots of these crops using culture dependent methods. The most commonly found endophytic bacteria associated with the crops included *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Stenotrophomonas*. The objective of the current study was to assess the potential use of these bacterial endophytes to promote plant growth. The effect of bacterial endophytes on seed germination, root elongation, plant growth and nutrient uptake was assessed on canola, wheat, pea and lentil in laboratory and growth chamber studies.

5.2. Abstract

Bacterial endophytes are detectable within plants tissues without showing external signs of infection. These bacteria can influence plant growth by synthesizing plant growth regulators, facilitating nutrient uptake from the soil and/or limiting the growth of phytopathogens that would affect plant growth and productivity. Recent studies also indicated that the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is a key mechanism involved in the promotion of plant growth under stress conditions. Therefore, the potential use of bacterial endophytes may increase the productivity of crops in agricultural ecosystems. The objectives of this study were: *i*) to assess the plant growth promotion potential of bacterial endophytes inoculated in canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.); and *ii*) to determine the production of ACC deaminase by candidate bacterial endophytes. A total of 157 bacterial endophytes were tested for their effect on seed germination which varied from seed germination promotion to inhibition. Forty isolates that caused the highest germination rates of the host crop seeds were tested for root elongation and ACC deaminase production. Nine bacterial endophytes stimulated root elongation in canola and wheat. The ACC deaminase activity was detected in 16 bacterial strains, of which only six strains increased root elongation. This result suggests that multiple bacterial mechanisms may be involved in the stimulation of root elongation in these crops. Inoculation with strains WCB1_23 (*Agrococcus carbonis*), WCB2_14 (*Stenotrophomonas rhizophila*), WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*) and WK1_6 (*Xanthomonas fuscans*) promoted shoot growth in canola grown in agricultural soils at flowering. Some of these strains also produced an increase in the nitrogen and sulfur content in the shoot of canola. Bacterial strain CM3_1 (*Stenotrophomonas maltophilia*) did not stimulate shoot growth, but increased nitrogen and sulfur content in the shoot of wheat. These results indicated that inoculation with selected bacterial endophytes may enhance canola growth.

5.3. Introduction

Effective management of crops usually requires intensive application of chemical fertilizers, tillage, irrigation and pesticides (Foley et al., 2005). However, the implementation of these practices sometimes has negative effects on the long-term soil productivity and the environment. In this context, beneficial microorganisms associated with crops are important for the establishment and development of agricultural ecosystems (Van Elsas et al., 2006). A diverse group of microorganisms termed endophytes, can inhabit the internal plant tissues causing no visible harm to the host plant (Hallmann et al., 1997). Among these microorganisms, endophytic bacteria may exhibit physiological characteristics that improve nutrient acquisition and/or limit the proliferation of phytopathogens. The most commonly found genera of bacterial endophytes are *Bacillus*, *Burkholderia*, *Microbacterium*, *Micrococcus*, *Pantoea*, *Pseudomonas* and *Stenotrophomonas* (Santoyo et al., 2016). These genera are also common inhabitants of the rhizosphere, thus suggesting that the plant endosphere may be a subset of the rhizosphere inhabiting bacteria (Germida et al., 1998; Marquez-Santacruz et al., 2010). However, as compared to the bacterial communities of the rhizosphere and/or on the rhizoplane, bacterial endophytes may establish closer associations with a host plant (Beattie, 2007). Consequently, the beneficial effects of endophytes to their host plants are in general greater than those of rhizobacteria, making the application of endophytic bacteria a promising tool for crop growth (Compant et al., 2010; Ma et al., 2011).

Bacterial endophytes have the potential to benefit plant growth by increasing nutrient availability, phytostimulation and biocontrol activity (Bloemberg and Lugtenberg, 2001; Gaiero et al., 2013). In addition, endophytic bacteria may contribute to maintaining agricultural production under unfavorable environmental conditions, such as drought, extreme soil temperature, soil salinity, as well as pathogens and pests (Glick, 2015). Therefore, endophytes have great potential to become effective microbial inoculants that can be used to enhancing crop growth. The formulation of inoculants requires knowledge on the ability of bacteria to colonize, multiply and persist in the host crop, as well as their ability to adapt to the various biotic and abiotic conditions commonly prevalent in agricultural fields (Finkel et al., 2017). Usually, this process involves *in vitro* laboratory tests, followed by microcosm, greenhouse and field studies.

Several physiological mechanisms are involved in the plant growth promotion by bacterial endophytes including: (*i*) biological N_2-fixation nitrification and ammonia oxidation, (Maier and Triplett, 1996; James 2000; Elbeltagy 2001; Cocking 2003; Sessitsch et al., 2012), (*ii*) solubilization of soil minerals such as phosphorus (Oteino et al., 2015; Borah et al., 2017), (*iii*) the production of siderophores (Loaces et al., 2011; Sessitsch et al., 2012; Abbamondi et al., 2016) and (*iv*) the synthesis of phytohormones (Liu et al., 2017). Recent studies also indicated that the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is a key mechanism involved in the promotion of plant growth (Glick, 2014). This enzyme cleaves ACC (the immediate precursor of ethylene in plants) into ammonia and α-ketobutyrate, and it has been produced by soil bacteria and fungi (Glick et al., 2015). The ethylene is detected in all higher plants and is involved in the modulation of plant growth and development processes (Glick et al., 1998). However, in high concentrations, ethylene may inhibit plant growth or even causes death (Glick et al., 2007). Ethylene production may increase in response to both biotic and abiotic processes (Glick et al., 1998). Thus, by breaking down some of the ACC in plants, microbes containing the ACC deaminase enzyme can potentially lower plant ethylene levels thereby regulating ethylene inhibition on plant growth (Glick, 2015). Inoculation of plants with ACC deaminase producing bacteria can increase plant growth when plants are exposed to abiotic and biotic stress conditions (Wang et al., 2000; Mayak et al. 2004; Reed and Glick, 2005; Arshad et al., 2008; Ali et al., 2012; Glick 2012; Li et al., 2013). However, it is still unclear which are the main mechanisms that control the growth promoting potential of bacterial endophytes and their effect on plant growth.

The previous study (Chapter 3), assessing the diversity of root associated bacteria with canola, wheat, pea and lentil grown in agricultural fields from Saskatchewan, Canada, isolated and identified bacterial strains using cultivation methods. Most common endophytic bacteria associated to the studied crops included *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Stenotrophomonas*. In the current study, it was hypothesized that bacterial endophytes isolated from crops have potential to promote plant growth. The main objective of the study was to assess the plant growth promotion potential of bacterial endophytes inoculated to canola, wheat, pea and lentil. Production of ACC deaminase by selected bacterial endophytes was assessed *in vitro* in laboratory studies.

5.4. Materials and Methods

5.4.1. Experimental Design

In this study, a total 157 bacterial endophytes isolated from the roots of canola, wheat, pea and lentil grown in agricultural soils in Saskatchewan were tested for growth promotion capacity. These isolates were assessed for ability to stimulate the germination of canola, wheat, pea and lentil seeds. Based on the results, ten isolates from each crop were selected to analyze their effect on root elongation of the same crops. The combinations of crops and isolates that promoted root elongation were tested on plants grown in two agricultural soils in growth chamber studies. Additionally, the ACC deaminase activity of isolates that promoted seed germination was tested.

5.4.2. Inoculum Preparation

Bacterial endophytes strains used in this study were stored in a 1:1 mixture ($v{\cdot}v^{-1}$) of 1/10 Tripticase soy broth (TSB) and glycerol at -80°C, in the culture collection of Soil Microbiology Laboratory of the University of Saskatchewan. A growth culture of each isolate was initiated on 1/10 TSA plates and incubated at 28ºC for 72 h. Fresh individual colonies of each isolate were transferred to 300-mL (500-mL erlenmeyer flasks) of autoclaved liquid ½ Trypticase Soy Broth (TSB) medium and cultured on a rotary shaker (120 rpm) at 28°C for 48 h. At that time, the optical density (OD) of the cell suspensions was measured and adjusted to OD=1 with autoclaved phosphate buffered saline (PBS) to a final volume of 150 mL. Bacterial suspensions were concentrated by centrifugation (15 min at 5000 × g), washed three times with 150 mL in PBS, and re-suspended in 20 mL of sterile tap water. Inoculum cell concentration was measured by serially diluting the resulting cell suspension, spread on 1/10 TSA plates and incubated at 28°C for 72 hrs. After that time, the number of colony-forming units (CFU) was determined. The procedure yielded approximately 10^{10} $CFU{\cdot}mL^{-1}$ of inoculum.

5.4.3. Seed Inoculation

Seeds were surface disinfected by soaking in ethanol (65% $v{\cdot}v^{-1}$) for 3 min and sodium hypochlorite (1.2% $v{\cdot}v^{-1}$) for 5 min, followed by 10 rinses in autoclaved tap water (Vincent 1970). Surface disinfected seeds (50 seeds) were mixed with 5 mL of bacterial suspension on a rotary shaker (120 rpm) at 28°C for 4 h, allowing the bacteria to penetrate into the seeds to ensure colonization during seed germination. Control seeds were mixed with 5 mL of autoclaved distilled

water. Seeds were placed in sterile plastic bags containing 1.5 mL of sterile 1% ($w \cdot v^{-1}$) methylcellulose, mixed with 7.5 g of talc and rolled until uniformly coated. This formulation was air dried overnight (12 h) in the biosafety cabinet. To check the bacterial colonization on the seeds, five coated seeds from each treatment were aseptically homogenized and the resulting suspension was serially diluted, spread on 1/10 TSA plates and incubated at 28°C for 72 hrs. The procedure yielded approximately 10^8 CFU·seed^{-1}. Preliminary experiments using seeds coated with a mixture autoclaved bacterial strains exhibited no significant differences on the seed germination rate of the studied crops compared to seeds coated with autoclaved distilled water.

5.4.4. Seed Germination Assay

The effect of bacterial isolates on seed germination was performed by placing coated seeds onto sterile filter paper moistened with 4 mL of autoclaved distilled water in Petri dishes and incubated at room temperature. Four replicate Petri dishes containing ten coated seeds each were used for each treatment and control. Germination (%) was estimated as the percentage of germinated seeds, after 40 h (day 2) and 184 h (day 8) of seed inoculation.

5.4.5. Root Elongation Assay

Root elongation assay was performed under gnotobiotic conditions using plastic seed-pack growth pouches (16.5 × 18 cm) containing chromatographic filter paper (Mega International, Minneapolis, MN, United States) as previously described by Belimov et al. (2002). Twenty milliliters of 1:5 Hoagland's nutrient solution were added to each pouch, which were wrapped with aluminum foil and sterilized in an autoclave at 120°C for 20 min. Ten coated seeds were placed inside the growth pouches and after germination only 5 plants were left to grow. Five replicate pouches were used for each treatment and control. Growth pouches were covered with aluminum foil to prevent light in plant roots and allowed to grow in a growth chamber with a 16 h/25°C day (1500 µmol·m^{-2}) and 8 h/15°C night cycle. The moisture content in the pouches was kept constant throughout the experiment by additions of sterile distilled and 1:5 Hoagland's nutrient solution on alternate days. These procedures were carried out under aseptic conditions using a biosafety cabinet to avoid contamination. Pea and lentil plants were harvested at 15 days, wheat plants at 12 days and canola plants at 7 days. The roots were scanned using an Epson (Perfection V700) scanner with a resolution of 600 dpi and the root length determined using WinRhizo 2013e (Regent Instruments, Canada).

5.4.6. Assessment of Endophytic Bacteria Inoculation on Crops Growing in Agricultural Soils

Soils were collected in two fields located in Central Butte (50°43'N, 106°25'W) corresponding to Brown Chernozem (FAO, 2018). Soil samples were sent to ALS Environmental Laboratory (Saskatoon, Saskatchewan) for basic soil analysis (Table 5.1). Protocols used for soil analysis are previously described in section 3.4.2 (chapter 3). Bulk soil was air dried, sieved (<2mm) and 1.5 kg transferred to 1.5 L plastic pots. Wheat (CDC Waskeda) and canola (Invigor L150) were grown in potted soils (n=4). Seeds were surface disinfected by soaking in ethanol (65% $v{\cdot}v^{-1}$) for 3 min and sodium hypochlorite (1.2% $v{\cdot}v^{-1}$) for 5 min, followed by 10 rinses in autoclaved tap water (Vincent 1970). Ten wheat or canola coated seeds were allowed to germinate, and then thinned so only 2 plants were left to grow. Plants were grown in a growth chamber with a 16 h/25°C day (1500 $\mu mol{\cdot}m^{-2}$) and 8 h/15°C night cycle. Each treatment and control pots were replicated four times. Pots were arranged in a randomized complete block design and rotated daily. The moisture content of the soil was kept at 50% gravimetric water content during the experiment. Plants were harvested at flowering stage and plant height and shoot fresh weight measured. Then, shoots were oven-dried at 60 °C for a week, dry biomass weighed and total nitrogen and sulfur determined. The total nitrogen and sulfur content was analyzed by furnance technology using an automated TruMac LECO and an SC832 LECO analyzers, respectively.

Table 5.1. Physical and chemical properties of soil samples collected at two Saskatchewan agricultural fields in Central Butte.

						Available			
	pH	Sand	Silt	Clay	OM	NO_3^-	SO_4^{2-}	PO_4^{3-}	K^+
		%				($mg{\cdot}kg^{-1}$ soil)			
Soil A	7.4	50	41	9	2.9	11.7	21.7	9.2	530
Soil B	6.8	71	13	16	1.1	22.2	22.0	5.6	212

5.4.7. 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Activity of Bacterial Isolates

To determine the production of ACC deaminase, isolates were cultured under ACC deaminase inducing conditions (Penrose and Glick, 2003). To induce ACC deaminase activity, cells were grown first in rich and then minimal ACC medium. First, bacterial isolates were grown in 5 mL (50-mL tubes) of autoclaved liquid ½ TSB medium and cultured on a rotary shaker (120 rpm) at 28°C for 24 h. After culturing in TSB, 100 µL of culture were transferred to 5 mL of autoclaved

minimal medium known as DF salts using $(NH_4)_2SO_4$ as nitrogen source and incubated for 24 h in a water bath on a rotary shaker (200 rpm) at 28°C. Then, 100 µL of culture were transferred to 5 mL DF salts using ACC (3.0 mM) as the sole nitrogen source and incubated for 1 h in a water bath on a rotary shaker (200 rpm) at 28°C. Finally, to observe which isolates contained ACC deaminase, cultures were streaked on DF salts agar plates that contained ACC (30 µmol·plate^{-1}) and incubated at 28°C for 7 days.

5.4.8. Statistical Analyses

Seed germination rates, root elongation, root fresh weight, shoot length, shoot weight and nitrogen and sulfur contents were subjected to analysis of variance (ANOVA) and Dunnett's post hoc test using SAS software version 9.6 (Copyright © 2002-2010 SAS Institute Inc. Cary, NC, USA.).

5.5. Results

5.5.1. Seed Germination Assay

In order to detect compatibility with the crops, a total of 157 bacterial endophytes isolated from canola (42), wheat (63), pea (23) and lentil (29) were tested for their effect on seed germination. In general, most bacteria had no significant effect ($P \leq 0.05$) on seed germination of the four crops (Tables D1-D4, Appendix D). A higher number of bacteria exhibited significant effect ($P \leq 0.05$) on the seed germination rates at day 2, compared to day 8 (Figs. 5.1 and 5.2). The number of isolates exhibiting a positive effect ($P \leq 0.05$) on the germination rates at day 2 (42 isolates) and day 8 (20 isolates) was higher in canola, when compared to other crops. In lentil, seed germination at day 8 was significantly increased ($P \leq 0.05$) by 16 isolates, but only three bacterial isolates promoted seed germination at day 2. In contrast, the number of isolates that significantly promoted ($P \leq 0.05$) seed germination was lower for wheat and pea, compared to other crops.

Interestingly, many bacteria inoculants significantly inhibited ($P \leq 0.05$) lentil seed germination at day 2 (26 isolates) and day 8 (8 isolates), most of these strains originally isolated from wheat roos (Figs. 5.1 and 5.2). Similarly, seed germination at day 2 in pea was significantly inhibited ($P \leq 0.05$) by 39 isolates, and six bacterial isolates inhibited seed germination at day 8. In contrast, seed germination at day 2 in canola was significantly ($P \leq 0.05$) inhibited by nine isolates, but inhibition of seed germination at day 8 did not occur. In addition, seed germination at day 2 in

wheat was significantly ($P \leq 0.05$) inhibited by 10 isolates, and only 2 bacterial isolates inhibited seed germination at day 8.

Based on their overall effects on the seed germination rates, a total of 40 endophytes were selected for further studies. From this, ten bacterial endophytes from each crop of origin that exhibited the highest seed germination rates were selected (Tables D1-D4, Appendix D). For example, selected bacterial endophytes originally isolated from canola belonged to the genera *Bacillus, Pantoea, Pseudomonas, Pseudoxanthomonas* and *Stenotrophomonas*. Strains isolated from wheat belonged to the genera *Agrococcus, Brevibacillus, Galbitalea, Leifsonia, Microbacterium, Paenibacillus, Stenotrophomonas* and *Xanthomonas*. Isolates from pea corresponded to the genera *Bacillus, Pantoea, Pseudomonas, Rhizobium* and *Stenotrophomonas*. Finally, isolates from lentil belonged to the genera *Bacillus, Pantoea, Pseudomonas, Rhizobium* and *Stenotrophomonas*.

5.5.2. Root Elongation Assay

Root elongation assay was assessed using 40 isolates that exhibited the highest promoting effect on seed germination (Tables D1-D4, Appendix D). Isolates WCB1_23 (*Agrococcus carbonis*), WM1_7 (*Leifsonia xyli*), WCB2_2 (*Paenibacillus taohuashanense*), CS1_1 (*Pantoea vagans*), PM1_1 (*Pseudomonas* sp.), WCB1_10 (*Rhodococcus cerastrii*), WCB2_14 (*Stenotrophomonas rhizophila*) and WK1_6 (*Xanthomonas fuscans*) significantly increased ($P \leq 0.05$) the root length in canola by 23 to 37% compared with control (Fig. 5.3). Similarly, the isolate CM3_1 (*Stenotrophomonas maltophilia*) also increased ($P \leq 0.05$) the root length in wheat by 14% compared with control (Fig. 5.4). In contrast, isolates LK1_4 and PS1_11 (*Bacillus halosaccharovorans*), LSV2_18 (*Microbacterium murale*) and LCB1_3 (*Pantoea agglomerans*) inhibited ($P \leq 0.05$) root elongation in canola by 32 to 39% compared with control, whereas no inhibition effect ($P \leq 0.05$) was detected in wheat (Figs. 5.3 and 5.4). No significant effect ($P \leq 0.05$) on root elongation was observed in pea or lentil (Fig. 5.4). Based on their overall effects on the root elongation of the crops, a total of nine endophytic bacteria that stimulated root growth in canola and wheat were selected for further studies.

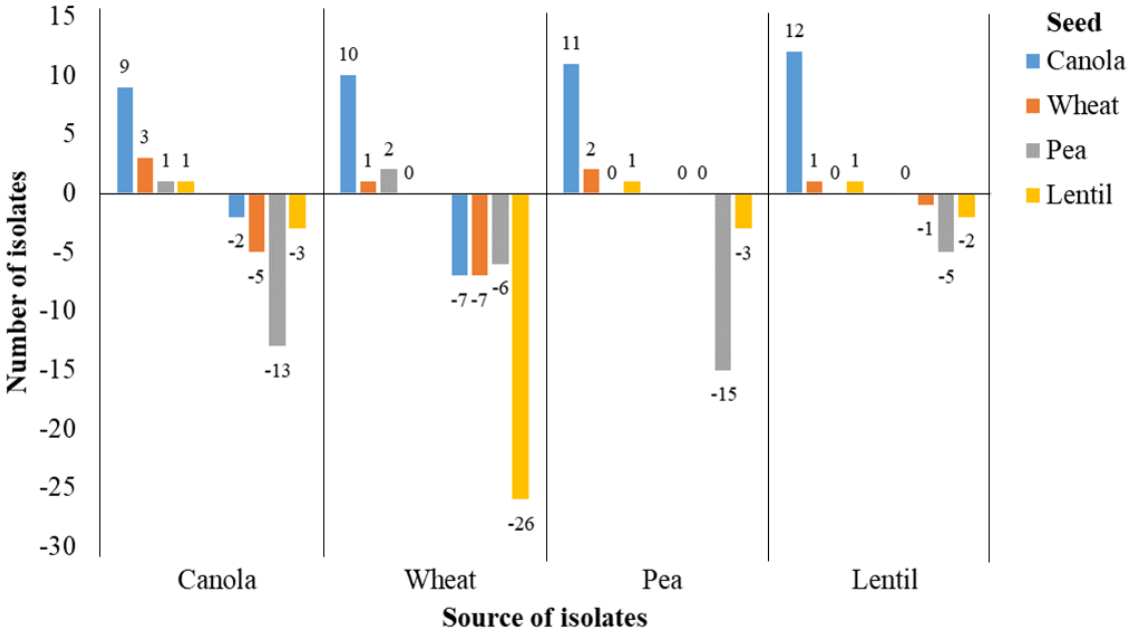

Fig. 5.1. Number of bacterial isolates promoting (positive values) or inhibiting (negative values) seed germination at day 2 of canola, wheat, lentil and pea, compared with control uninoculated seeds; Dunnett, P≤0.05.

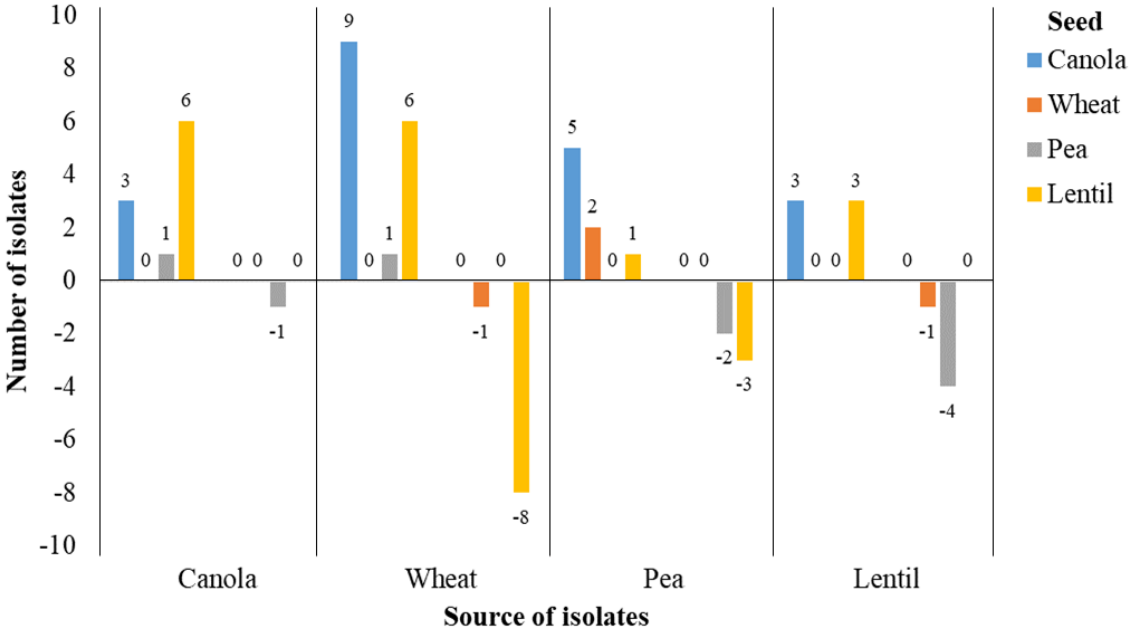

Fig. 5.2. Number of bacterial isolates promoting (positive values) or inhibiting (negative values) seed germination at day 8 of canola, wheat, lentil and pea, compared with control uninoculated seeds; Dunnett, P≤0.05.

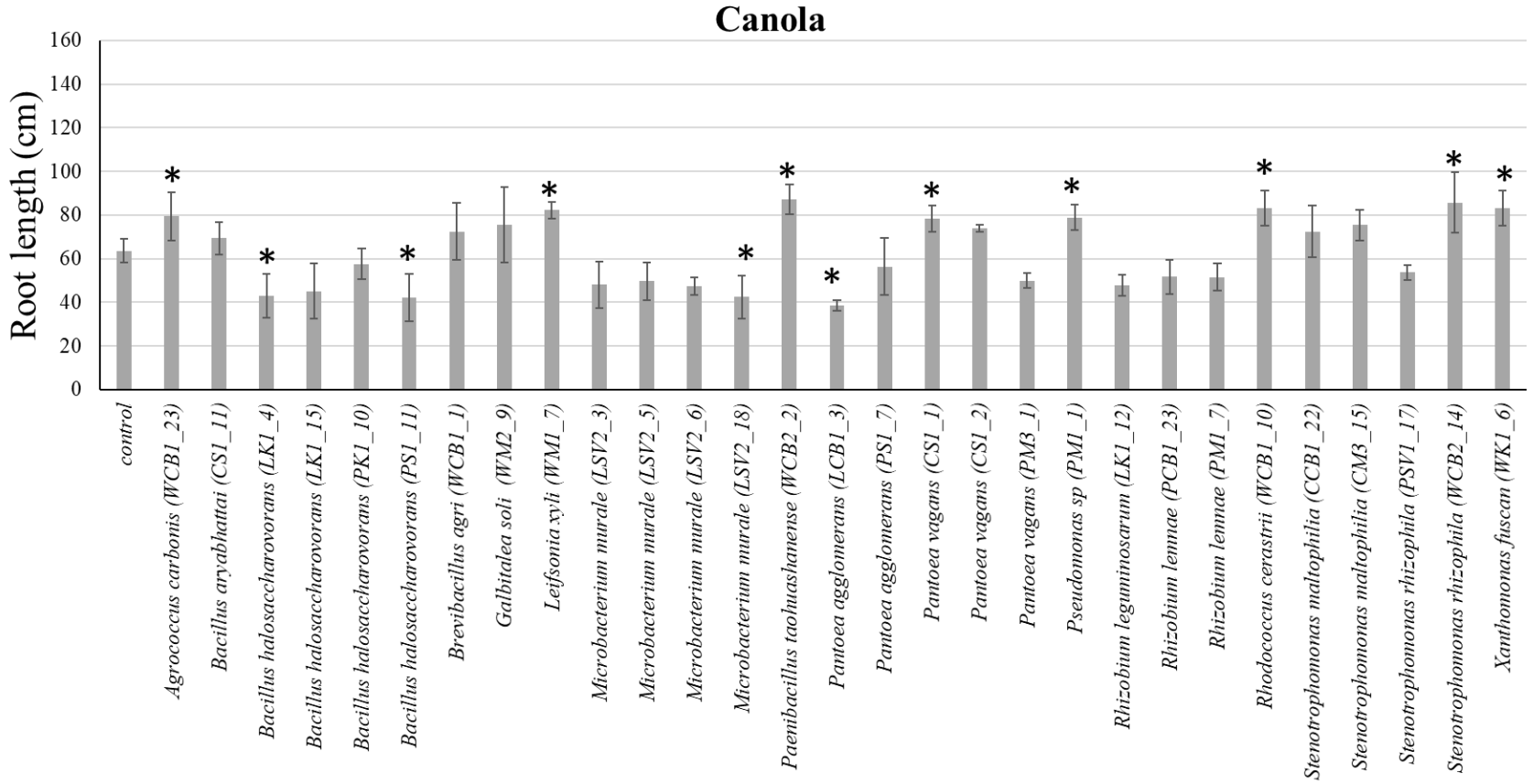

Fig. 5.3. Root elongation (cm) of canola inoculated with root bacterial endophytes. Error bars represent standard deviation (n=20). Asterisks (*) indicate significant difference compared with control; Dunnett, $P \leq 0.05$.

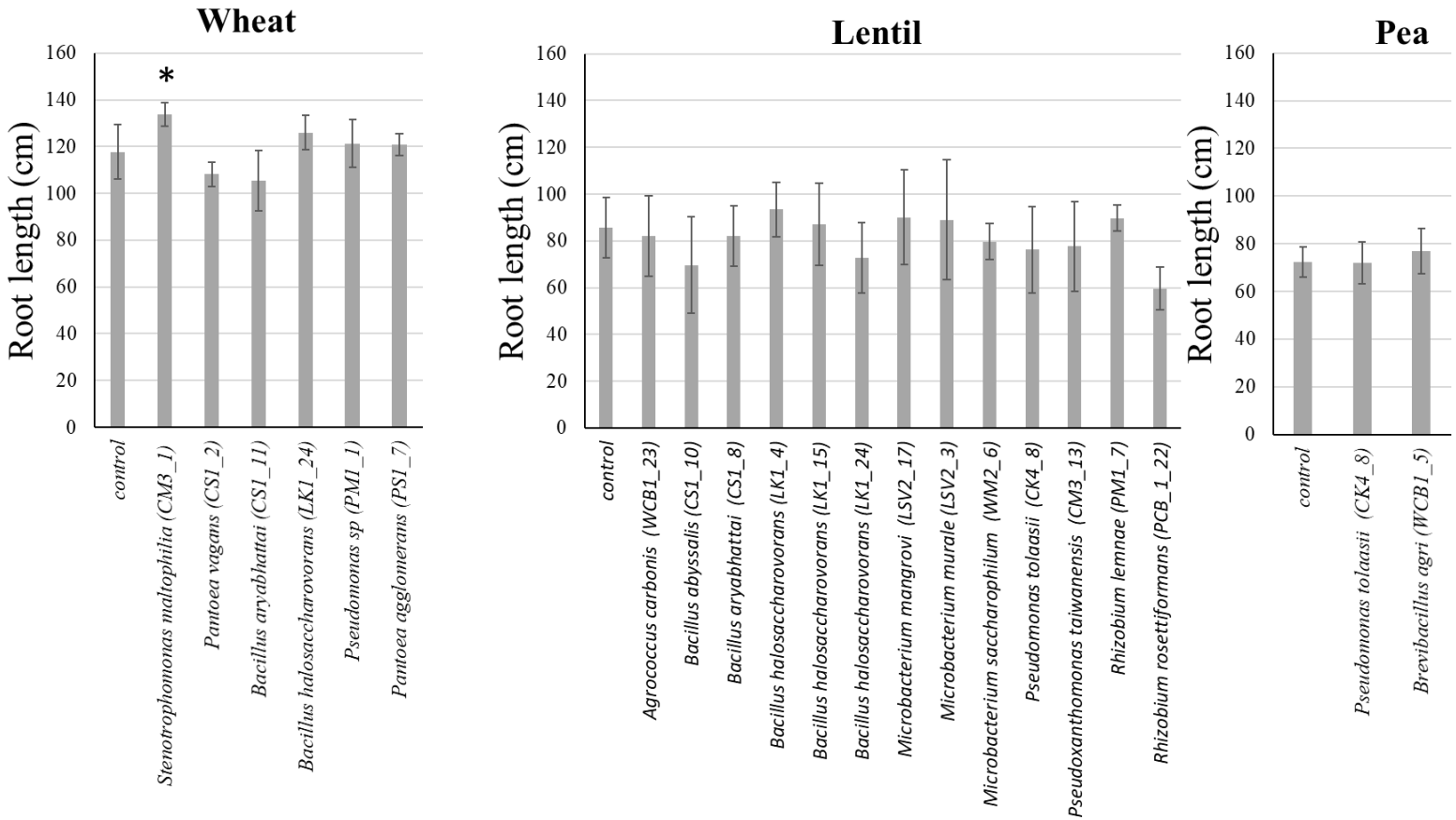

Fig. 5.4. Root elongation (cm) of wheat, lentil and pea inoculated with root bacterial endophytes. Error bars represent standard deviation (n=20). Asterisks (*) indicate significant difference compared with control; Dunnett, $P \leq 0.05$.

5.5.3. Effect of Bacterial Endophytes Inoculation on Wheat and Canola Grown in Agricultural Soils

Based on seed germination and root elongation results, nine endophytic bacterial strains were tested for plant growth promotion of wheat and canola grown in two agricultural soils collected at Central Butte, Saskatchewan, Canada. These agricultural soils differed in some physical and chemical properties. For example, soil A had a higher available potassium and organic matter content as well as a lower available nitrogen, compared to soil B (Table 5.1). Soils significantly influenced the effect of bacterial inoculants on shoot dry weight ($P < 0.0001$) and nitrogen content of shoot of wheat ($P = 0.002$) (Table 5.2). Similarly, soils significantly influenced the shoot fresh and dry weight ($P < 0.0001$), shoot length ($P < 0.0001$) and nitrogen content in the shoot ($P < 0.0001$) of canola (Table 5.2). Bacterial inoculation of wheat and canola had a significant effect on all assessed plant growth parameters, except for the shoot length (Table 5.2, Figs. 5.5-5.9). The nitrogen concentration in the plant shoots was up to 8.5 and 9.3 $g \cdot kg^{-1}$ dry weight in canola and wheat, respectively (Fig. 5.8). In addition, the sulfur concentration in the plant shoots was up to 4.1 and 1.4 $g \cdot kg^{-1}$ dry weight in canola and wheat, respectively (Fig. 5.9).

Inoculation of wheat with strain CM3_1 (*Stenotrophomonas maltophilia*) had no significant effect on any growth parameters assessed on the plants in soil B (Fig. E.2, Appendix E). In contrast, wheat plants grown in soil A inoculated with strain CM3_1 (*Stenotrophomonas maltophilia*), exhibited a decrease ($P \leq 0.05$) in the shoot fresh and dry weight compared with control (Figs. 5.5 and 5.6) (Fig. E.1, Appendix E). However, these plants exhibited an increase ($P \leq 0.05$) in the nitrogen and sulfur content of the shoots, compared with control (Fig. 5.8 and 5.9).

Inoculation of canola with strains, WCB1_23 (*Agrococcus carbonis*), WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*) and WCB2_14 (*Stenotrophomonas rhizophila*) led to a significant increase ($P \leq 0.05$) in shoot fresh weight in plants grown in soil A compared with control (Figs. 5.5, 5.10-5.13). In addition, strain WK1_6 (*Xanthomonas fuscans*) increased shoot dry weight of canola compared with control (Fig. 5.6). In contrast, strains, WCB2_2 (*Paenibacillus taohuashanense*), PM1_1 (*Pseudomonas* sp.) and WCB1_10 (*Rhodococcus cerastrii*) had no significant effect on shoot growth in canola grown in soil A compared with control (Figs. 5.5-5.7) (Figs. D.3-D.5, Appendix D). Inoculation with strains WCB1_10 (*Rhodococcus cerastrii*), WCB2_14 (*Stenotrophomonas rhizophila*) and WK1_6 (*Xanthomonas fuscans*) significantly increased ($P \leq 0.05$) the nitrogen content in the shoot of canola grown in soil A compared with

control (Figs. 5.8). Interestingly, inoculation with strain WCB1_10 (*Rhodococcus cerastrii*) decreased ($P \leq 0.05$) the sulfur content in the shoot of the same plants (Fig. 5.9).

Inoculation of canola with endophytic bacterial strains had no significant effect ($P \leq 0.05$) on shoot growth in soil B (Figs. 5.5-5.7) (Figs. E.6-E.13, Appendix E). However, inoculation with strains WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*), PM1_1 (*Pseudomonas* sp.) and WCB1_10 (*Rhodococcus cerastrii*) significantly increased ($P \leq 0.05$) the nitrogen content in the shoot of canola grown in soil B compared with control (Fig. 5.8). In addition, strains WM1_7 (*Leifsonia xyli*) and WCB1_10 (*Rhodococcus cerastrii*) also increased ($P \leq 0.05$) the sulfur content in the shoot of these plants compared with control (Fig. 5.9).

Table 5.2. ANOVA of shoot fresh weight (SFW), shoot dry weight (SDW), shoot length (SL), nitrogen concentration (N) and sulfur concentration (S) of wheat and canola grown in two agricultural soils from Central Butte, Saskatchewan and inoculated with bacterial endophytes.

Wheat					
Source of variation	SFW	SDW	SL	N	S
Inoculant	0.004 ***	0.003 ***	0.4 n.s.	0.01 *	0.01 **
Soil	0.1 n.s.	<0.0001 ***	0.9 n.s.	0.002 **	0.2 n.s.
Inoculant × Soil	<0.0001 ***	0.05 *	0.6 n.s.	0.5 n.s.	0.3 n.s.
Canola					
Source of variation	SFW	SDW	SL	N	S
Inoculant	<0.0001 ***	0.01 **	0.3 n.s.	<0.0001 ***	<0.0001 ***
Soil	<0.0001 ***	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.2 n.s.
Inoculant × Soil	<0.0001 ***	0.0010 ***	<0.0001 ***	<0.0001 ***	<0.0001 ***

Note: *, **, ***, significant at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., not significant.

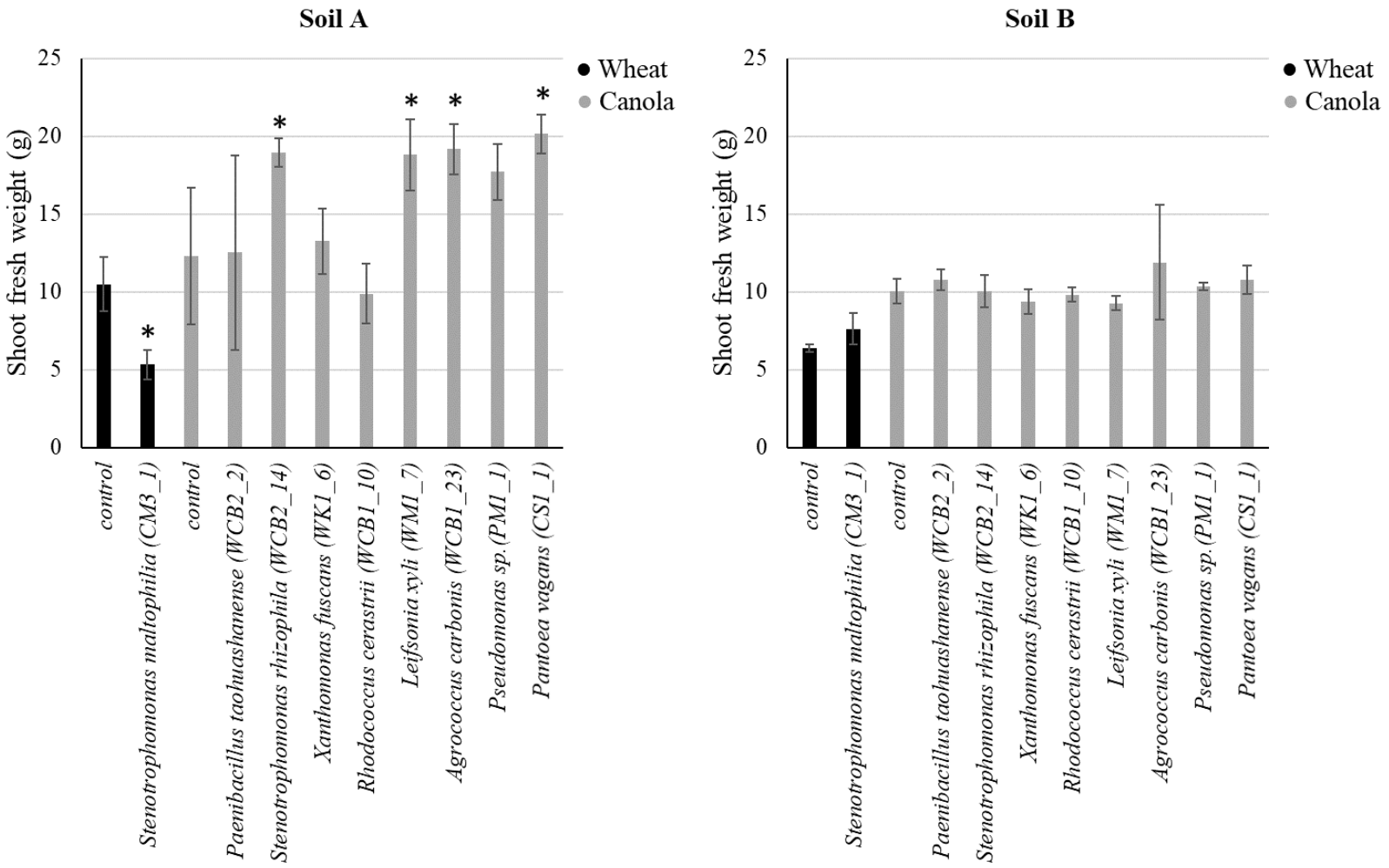

Fig. 5.5. Shoot fresh weight (g) of wheat and canola inoculated with endophytic bacteria and grown in two agricultural soils from Central Butte, Saskatchewan (Soil A and B). Error bars represent standard deviation (n=4). Asterisks (*) indicate significant difference compared with control; Dunnett, $P \leq 0.05$.

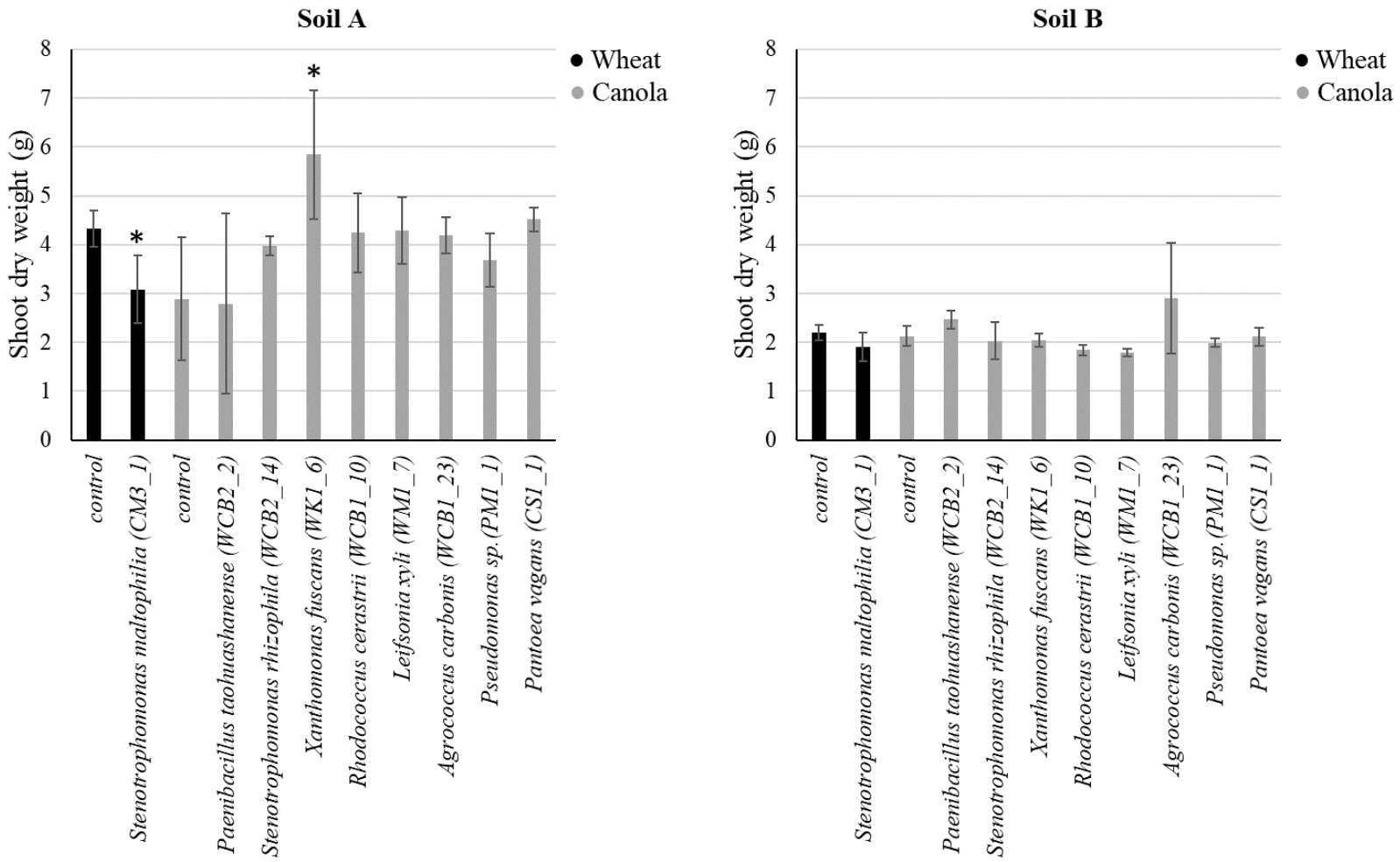

Fig. 5.6. Shoot dry weight (g) of wheat and canola inoculated with endophytic bacteria and grown in two agricultural soils from Central Butte, Saskatchewan (Soil A and B). Error bars represent standard deviation (n=4). Asterisks (*) indicate significant difference compared with control; Dunnett, $P \leq 0.05$.

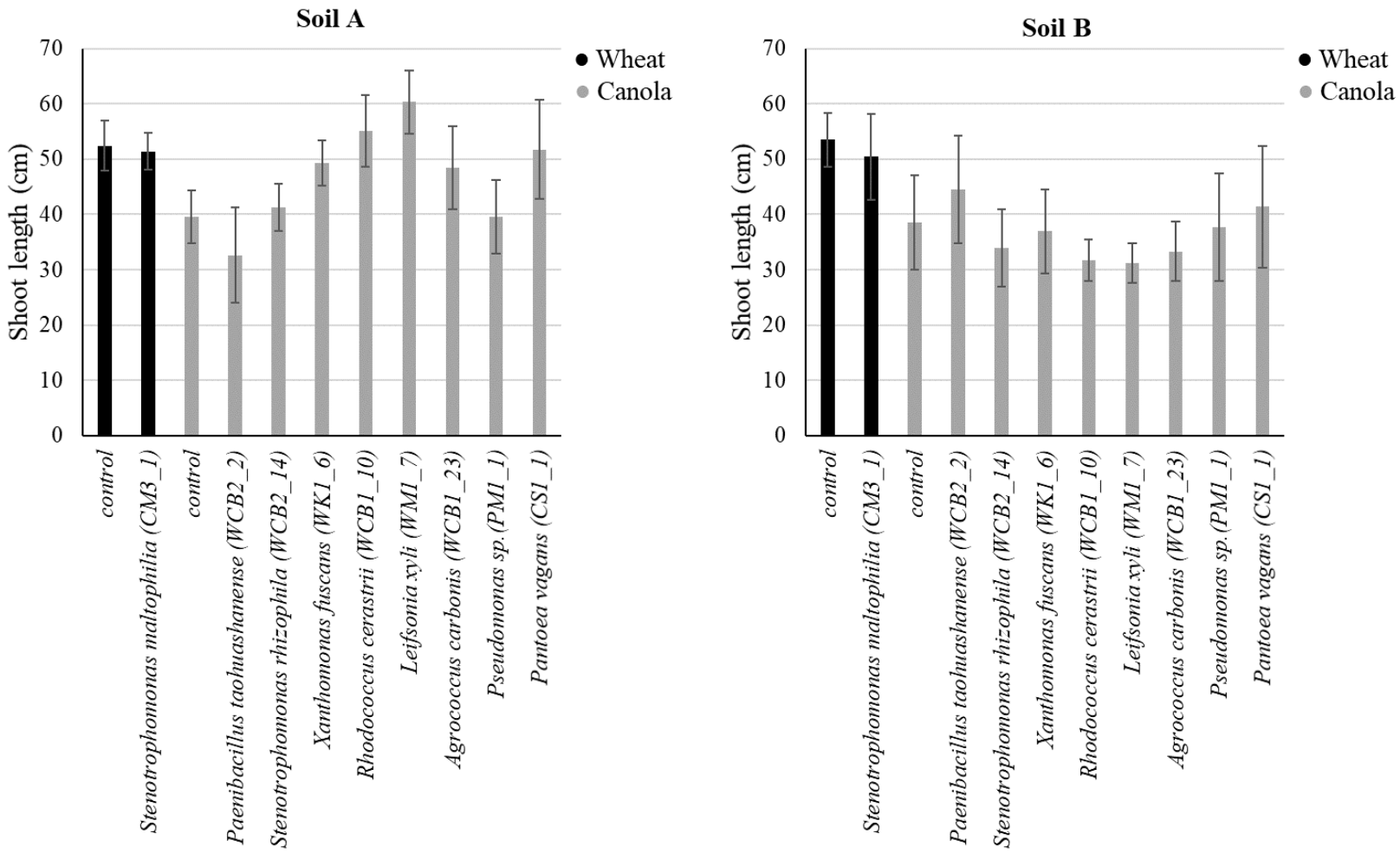

Fig. 5.7. Shoot length (cm) of wheat and canola inoculated with endophytic bacteria and grown in two agricultural soils from Central Butte, Saskatchewan (Soil A and B). Error bars represent standard deviation (n=4). Asterisks (*) indicate significant difference compared with control; Dunnett, $P \leq 0.05$.

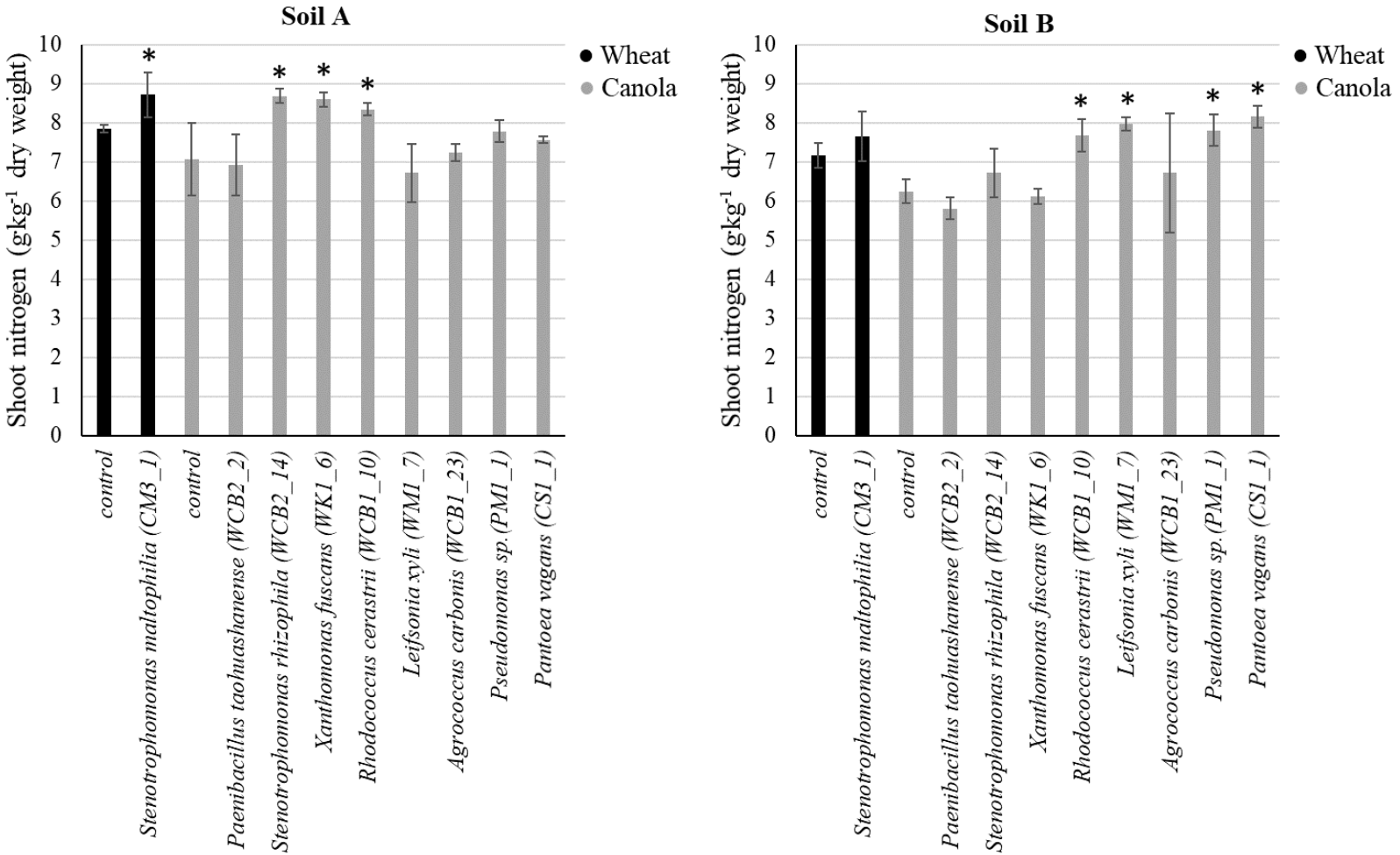

Fig. 5.8. Nitrogen concentration (g·kg^{-1} dry weight) in the shoot of wheat and canola inoculated with endophytic bacteria and grown in two agricultural soils from Central Butte, Saskatchewan (Soil A and B). Error bars represent standard deviation (n=4). Asterisks (*) indicate significant difference compared with control; Dunnett, P≤0.05.

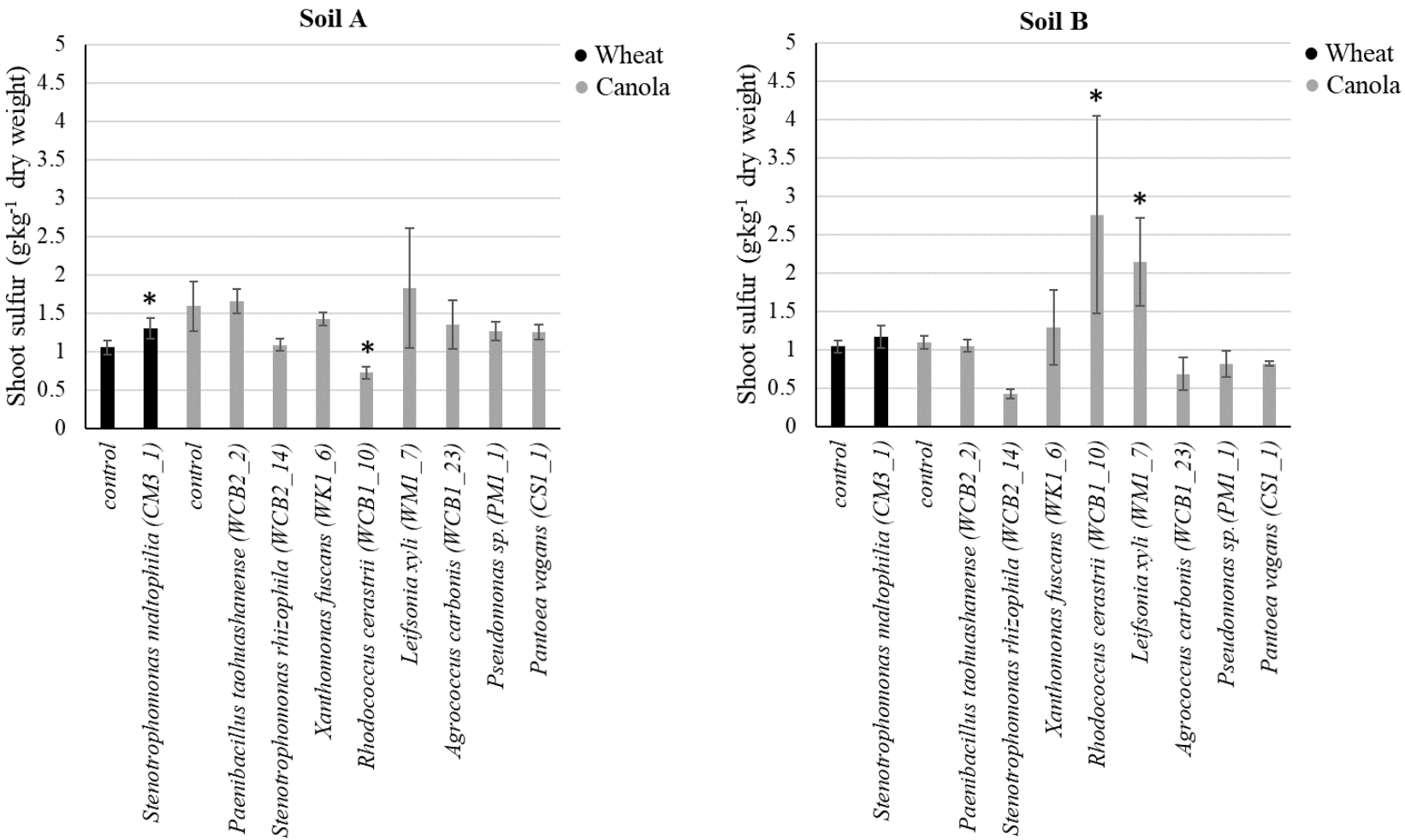

Fig. 5.9. Sulfur concentration (g·kg^{-1} dry weight) in the shoot of wheat and canola inoculated with endophytic bacteria and grown in two agricultural soils from Central Butte, Saskatchewan (Soil A and B). Error bars represent standard deviation (n=4). Asterisks (*) indicate significant difference compared with control; Dunnett, P≤0.05.

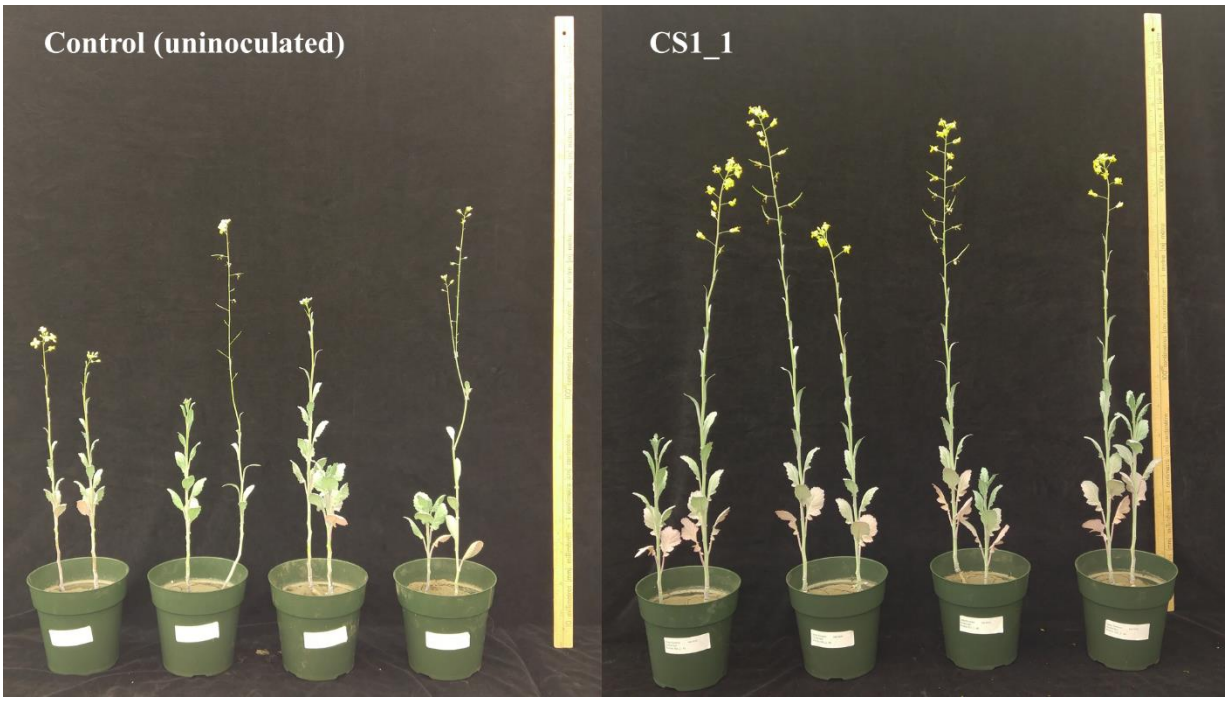

Fig. 5.10. Effect of *Pantoea vagans*, strain CS1_1 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

Fig. 5.11. Effect of *Agroccoccus carbonis*, strain WCB1_23 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

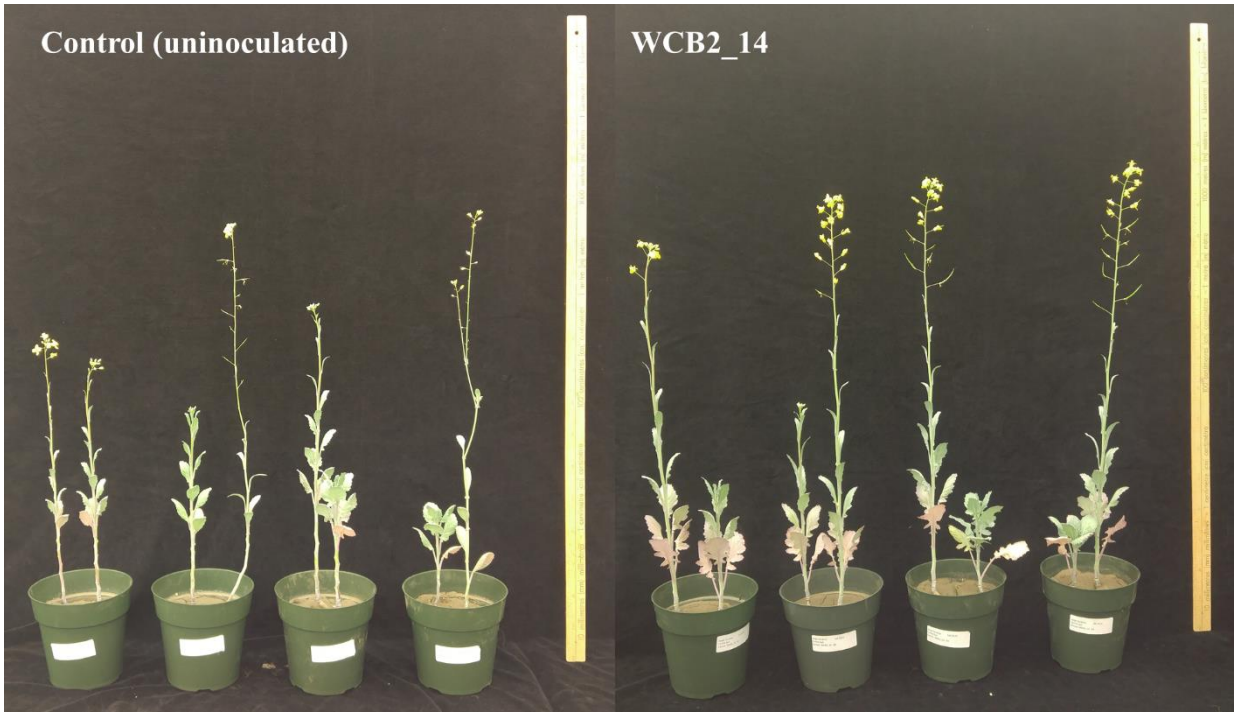

Fig. 5.12. Effect of *Stenotrophomonas rhizophila*, strain WCB2_14 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

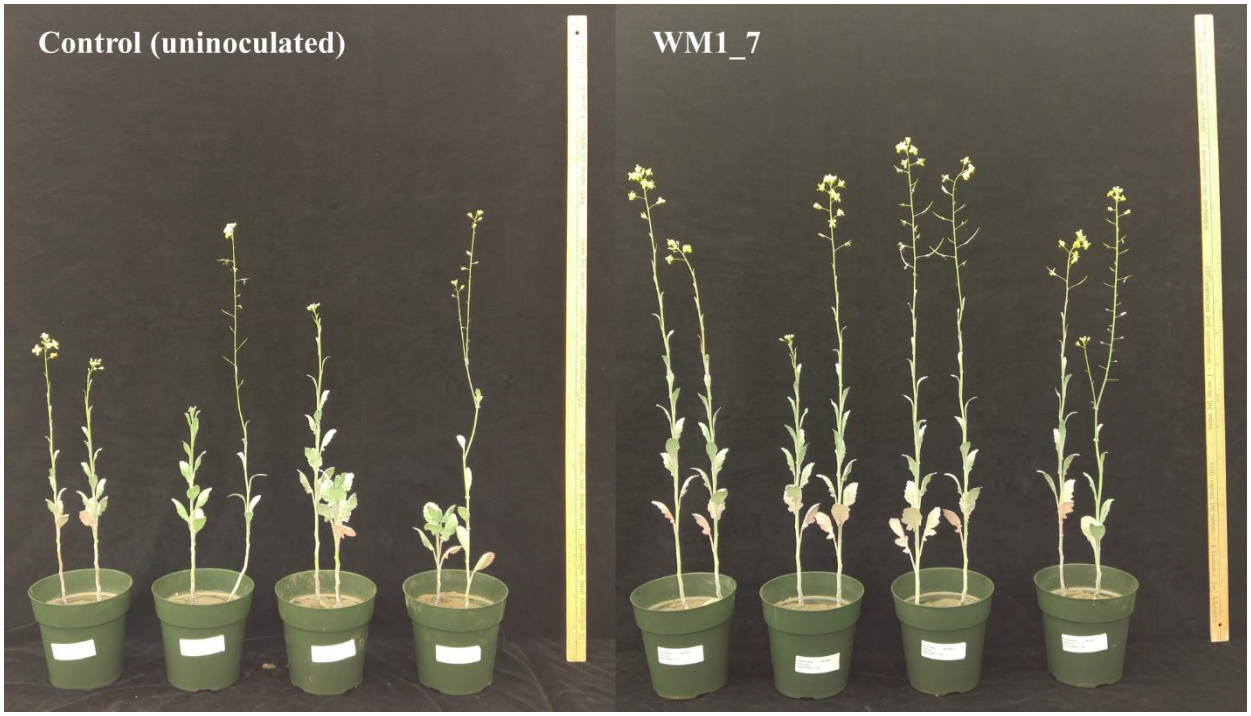

Fig. 5.13. Effect of *Leifsonia xyli*, strain WM1_7 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

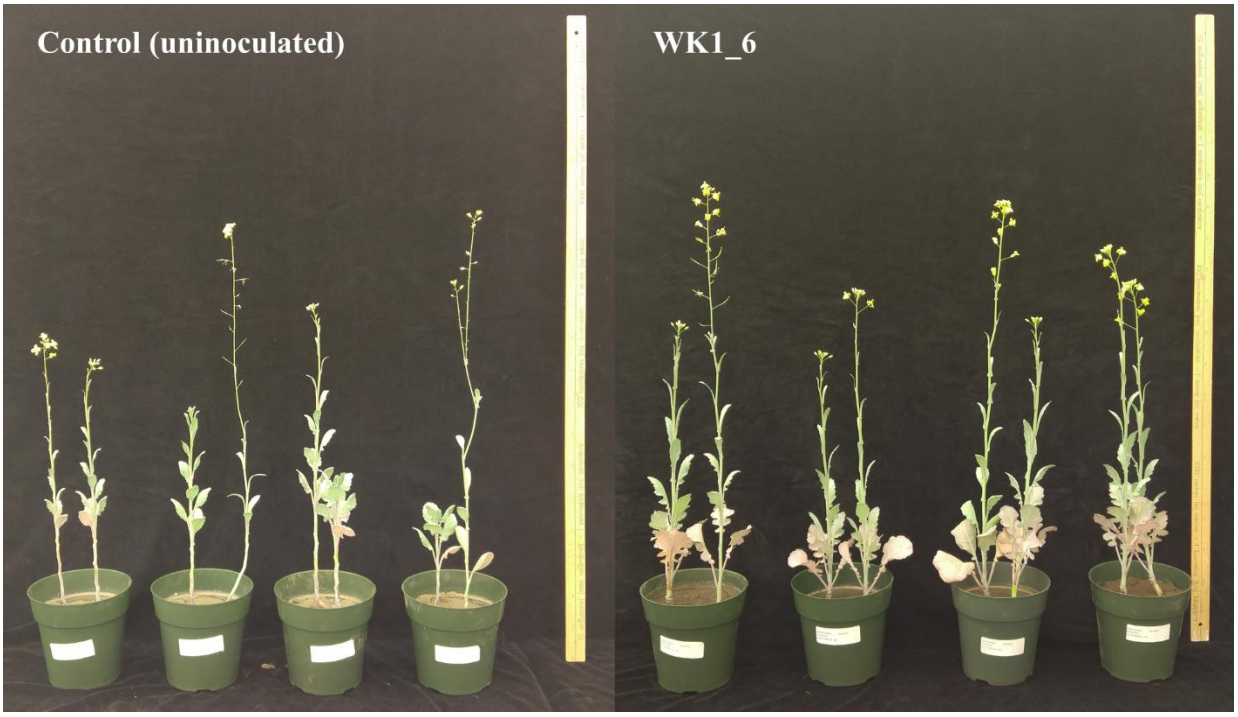

Fig. 5.14. Effect of *Xanthomonas fuscans*, strain WK1_6 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at floweing.

5.5.4. ACC Deaminase Activity of Bacterial Isolates

A total of 40 bacterial isolates that exhibited a positive effect on seed germination were tested for ACC deaminase activity (Table 5.3). A total of 16 isolates produced ACC deaminase activity under *in vitro* conditions. These isolates belonged to the genera: *Bacillus*, *Leifsonia*, *Pantoea*, *Pseudoxanthomonas*, *Rhodococcus*, *Stenotrophomonas* and *Xanthomonas*. Bacterial isolates CS1_1 (*Pantoea vagans*), CM3_1 (*Stenotrophomonas maltophilia*), WCB1_10 (*Rhodococcus cerastrii*), WCB2_14 (*Stenotrophomonas rhizophila*), WK1_6 (*Xanthomonas fuscans*) and WM1_7 (*Leifsonia xyli*) produced ACC deaminase and increased the root length of the host crops (Table 5.3, Figs. 5.3 and 5.4).

Table 5.3. ACC deaminase production by bacterial endophytes isolated from wheat, canola, lentil and pea plants. Isolates in bold exhibited a positive effect on root elongation.

Isolate	Classification	ACC
CS1_11	*Bacillus aryabhattai*	-
CS1_8	*Bacillus aryabhattai*	-
CS1_10	*Bacillus abyssalis*	-
CS1_2	*Pantoea vagans*	+
CS1_1	***Pantoea vagans***	**+**
CK4_8	*Pseudomonas tolaasii*	-
CM3_13	*Pseudoxanthomonas taiwanensis*	+
CM3_1	***Stenotrophomonas maltophilia***	**+**
CM3_15	*Stenotrophomonas maltophilia*	+
CCB1_22	*Stenotrophomonas maltophilia*	+
PK1_10	*Bacillus halosaccharovorans*	+
PS1_11	*Bacillus halosaccharovorans*	-
PS1_7	*Pantoea agglomerans*	+
PM3_1	*Pantoea vagans*	+
PM3_3	*Pantoea vagans*	+
PM1_1	***Pseudomonas* sp.**	**-**
PCB1_23	*Rhizobium lemnae*	-
PM1_7	*Rhizobium lemnae*	-
PCB1_22	*Rhizobium rosettiformans*	-
PCB1_17	*Stenotrophomonas rhizophila*	+

Isolate	Classification	ACC
WCB1_23	***Agrococcus carbonis***	**-**
WCB1_1	*Brevibacillus agri*	-
WCB1_5	*Brevibacillus agri*	-
WMF2_9	*Galbitalea soli*	-
WMF1_7	***Leifsonia xyli***	**+**
WMF2_6	*Microbacterium saccharophilum*	-
WCB2_2	***Paenibacillus taohuashanense***	**-**
WCB1_10	***Rhodococcus cerastrii***	**+**
WCB2_14	***Stenotrophomonas rhizophila***	**+**
WK1_6	***Xanthomonas fuscans***	**+**
LK1_15	*Bacillus halosaccharovorans*	-
LK1_4	*Bacillus halosaccharovorans*	-
LK1_24	*Bacillus halosaccharovorans*	-
LSV2_17	*Microbacterium mangrovi*	-
LSV2_3	*Microbacterium murale*	-
LSV2_5	*Microbacterium murale*	-
LSV2_6	*Microbacterium murale*	-
LSV2_18	*Microbacterium murale*	-
LCB1_3	*Pantoea agglomerans*	+
LK1_12	*Rhizobium leguminosarum*	-

5.6. Discussion

This study investigated the plant growth promotion potential of root bacterial endophytes that were isolated from roots of canola, wheat, lentil and field pea in a previous study (Chapter 3). As an initial screening for plant compatibility, a total of 157 isolates were inoculated to canola, wheat, lentil and pea seeds, some of which stimulated or inhibited seed germination. Previous study by Somova et al. (2001) also reported that germination of wheat seeds inoculated with *Pseudomonas putida* and *Pseudomonas fluorescens* were either stimulated, inhibited or remained at control levels depending on the bacterial numbers. Similarly, a study by Long et al. (2008) assessing the effect of bacterial endophytes from field-grown *Solanum nigrum* on plant growth of their host, as well as on another native plant, *Nicotiana attenuate,* also found that most bacterial strains stimulated seed germination, but one isolate inhibited seed germination. Furthermore, in the current study it was found that a higher number of isolates exerted positive effects on canola, whereas detrimental effects were detected more frequently in inoculated lentil or pea plants (Figs. 5.1 and 5.2). This result indicates that the effects of bacterial inoculants on seed germination were more pronounced in certain crops, independently of the host crop from where bacterial strains were originally isolated. Previous studies have reported that bacteria may increase seed germination by producing plant growth regulators, or by inducing seed physiological protection during stress conditions (Dodd et al., 2010; Mastouri et al., 2010). However, the stimulatory effect of plant regulators on seed germination only occurs at optimal levels of these compounds (Taiz and Zeiger, 2002). Thus, it is possible that high levels of phytothormones produced by an inoculant also may cause inhibition of seed germination (Miché et al., 2000). In addition, beneficial rhizobacteria such as *Azospirillum* sp. have been reported to secrete inhibitory molecules, which block the germination of striga seeds (Bouillant et al., 1997). Similarly, inhibition of seed germination by *Pseudomonas* spp. has been attributed to the production of hydrogen cyanide and phenazine (Kang et al., 2007). The potential beneficial, commensalistic or detrimental associations that may occur between the host seed and bacteria are also influenced by the seed exudates. These compouds are released by the seeds during the imbibition process and can modulate bacterial colonization and multiplication during early plant growth (Nelson, 2004).

The effect of 40 bacterial endophytes that exhibited the highest germination rates was assessed on root elongation of the crops. Results revealed that bacterial strains CS1_1 (*Pantoea vagans*), PM1_1 (*Pseudomonas* sp.), WCB1_10 (*Rhodococcus cerastrii*), WCB1_23 (*Agrococcus*

carbonis), WCB2_2 (*Paenibacillus taohuashanense*), WCB2_14 (*Stenotrophomonas rhizophila*), WK1_6 (*Xanthomonas fuscans*) and WM1_7 (*Leifsonia xyli*) significantly increased the root length of canola seedlings, compared to control plants (Fig. 5.3). Similarly, isolate CM3_1 (*Stenotrophomonas maltophilia*) increased the root length in wheat (Fig. 5.4). Previous studies also reported that bacterial species tested in the current study are able to stimulate root growth by synthetizing plant growth regulators. For example, Patten and Glick (2002) reported that indole acetic acid produced by *Pseudomonas putida* influenced the development of the root system in canola. Similarly, Afzal et al. (2015) reported that *Pantoea vagans* and *Pseudomonas geniculata* improved canola root growth under gnotobiotic conditions and produced indole acetic acid *in vitro*. In addition, Pallai et al. (2011) concluded the ability of *Pseudomonas fluorescens* to produce cytokinins may enhance root elongation and benefit canola seedling growth. Production of indole acetic acid and gibberellin by *Leifsonia xyli* also stimulated plant growth of tomato plants (Kang et al. 2017). Production of indoleacetic acid (IAA) also is detected in diazotrophic *Stenotrophomonas maltophilia* isolated from wheat, lettuce and rice (Park et al., 2005; Othman et al., 2013). Plant growth regulators produced by bacteria are known to influence cell division and root differentiation, thus leading to changes in the root architecture (Verbon and Liberman, 2016). The resulting enhancement of root growth produced by beneficial bacteria is advantageous for the establishment of plants as it increases their ability to anchor themselves to the soil and to obtain water and nutrients from their environment, thus improving their chances for survival (Patten and Glick, 2002). Therefore, promotion of root growth is one of the main parameters by which the beneficial effect of plant growth-promoting bacteria is measured (Glick et al., 1998).

In addition to the production of plant growth regulators by plant associated bacteria, other important microbial mechanism involved in plant growth promotion potential includes the production of ACC deaminase (Glick, 2014). In the current study, bacterial isolates CS1_1 (*Pantoea vagans*), CM3_1 (*Stenotrophomonas maltophilia*), WCB1_10 (*Rhodococcus cerastrii*), WCB2_14 (*Stenotrophomonas rhizophila*), WK1_6 (*Xanthomonas fuscans*) and WM1_7 (*Leifsonia xyli*) produced ACC deaminase and increased the root length of host crops (Table 5.3). This result suggests that promotion of root elongation in wheat and canola was correlated to the production of ACC deaminase by most of these endophytic bacteria. Previous studies also reported the production of ACC deaminase in strains of *Leifsonia* sp., *Pantoea* sp., *Rhodococcus* sp. and *Stenotrophomonas* sp. (Belimov et al., 2001; Madhaiyan et al., 2010; Zhang et al., 2011; Singh

and Jha, 2017). Bacteria containing the ACC deaminase enzyme are reported to potentially promote plant growth by lowering plant ethylene levels and thereby regulating ethylene inhibition on plant growth (Glick, 2015). However, in the current study, some bacterial strains that stimulated root growth were ACC deaminase negative (Table 5.3). These results suggest that production of ACC deaminase *in vitro* may not be the only mechanism involved in the promotion of root length in the studied crops. In fact, previous studies also found that some non-ACC deaminase bacteria can promote plant growth to a similar extent as ACC deaminase producing bacteria (Sheng et al., 2008; Afzal et al., 2015). Furthermore, Long et al. (2008) also concluded that a particular bacterial strain may affect plant growth and development using one or more microbial mechanisms.

Based on seed germination and root elongation results, nine endophytic bacterial strains were tested for plant growth promotion in wheat and canola grown in two agricultural soils (Soil A and B) collected in Central Butte, Saskatchewan, Canada. Visual characteristics of the plants suggest that canola and wheat were under stress during cultivation in both agricultural soils in the growth chamber, thus suggesting nutrient deficiency. In fact, canola plants (Figs. 5.5-5.9; Figs. E.3-E13, Appendix E) exhibited symptoms of phosphorus deficiency, as compared to plants grown in field conditions *e.g.*, presence of purple coloration in the older leaves (Canola Council of Canada, 2017; Horneck et al., 2011). Shoot nutrient analysis of both crops indicated N- and S-deficiency in both crops (Figs. 5.8 and 5.9) (Canola Council of Canada, 2017; American Agricultural Laboratory, 2019).

In the current study, the inoculation of wheat and canola with bacterial endophytes in potted agricultural soils had a significant effect on all assessed plant growth parameters, except shoot length (Table 5.2, Figs. 5.5-5.10). Bacterial strains WCB1_23 (*Agrococcus carbonis*), WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*) and WCB2_14 (*Stenotrophomonas rhizophila*) increased shoot fresh weight of canola (Fig. 5.5). Similarly, strain WK1_6 (*Xanthomonas fuscans*) increased shoot dry weigh of canola (Fig. 5.6). Previous studies also reported that some of the bacterial species tested in the current study were able to stimulate growth of agricultural crops. For example, Kang et al. (2017) reported that inoculation of tomato with *Leifsonia xyli* significantly enhanced amino acid content, improved total flavonoids, and increased phosphorus content, thus resulting in higher plant growth. Kang et al. (2014) also reported that a related *Leifsonia soli* promoted biomass, hypocotyl, and root length in seedlings of cucumbers (Kang et al., 2014). Similarly, *Pantoea vagans* increased growth, promoted root gravitropic response, stimulated root

hair formation, and protected rice seedlings from disease (Verma et al., 2018). Additionally, Bertrand et al. (2001) reported that inoculation of canola with strains of *Pseudomonas* sp. isolated from the rhizoplane and endorhizosphere increased the root dry weight. Belimov et al. (2001) reported that inoculation of pea with *Rhodococcus* sp. increased the root biomass. Berg at al. (2010) also reported that inoculation of wheat, tomato, lettuce, sweet pepper, melon, celery, and carrot with *Stenotrophomonas rhizophila* (strain DSM 14405) increased root and shoot length. In addition to plant growth promoting effects, some of bacterial isolates tested in the current study also are reported to cause diseases in agricultural crops. For example, *Leifsonia xyli* is the causal agent of ratoon stunting disease of sugarcane, whereas *Xanthomonas fuscans* causes the bacterial blight of bean (Darrasse et al., 2013; Young, 2017). Therefore, further studies of the impact of the inoculation of the endophytes tested in the current study must be performed before their extensive application in crop development.

Nitrogen is an essential nutrient for plant growth and development. Efficient nitrogen cycling influence agricultural ecosystem sustainability and is key to improving the crop production (Vinolas et al., 2001). Bacterial endophytes are important in the nitrogen cycling in agriculture and may reduce the need for chemical fertilizers (Maier and Triplett, 1996; James 2000; Elbeltagy 2001; Cocking 2003; Sessitsch et al., 2012). In the current study, inoculation of canola with strain CS1_1 (*Pantoea vagans*), WCB2_14 (*Stenotrophomonas rhizophila*), WCB1_10 (*Rhodococcus cerastrii*), WK1_6 (*Xanthomonas fuscans*), WM1_7 (*Leifsonia xyli*) and PM1_1 (*Pseudomonas* sp.) produced an increase in the nitrogen content in the shoot of canola (Fig. 5.8). Similarly, inoculation with strain CM3_1 (*Stenotrophomonas maltophilia*) significantly increased the nitrogen content in the shoots of wheat, compared to control plants (Fig. 5.8). Previous studies identified that some of the bacteria tested in the current investigation exhibit microbial mechanisms involved in the nitrogen cycling. For example, *Stenotrophomonas maltophilia* isolated from agricultural crops was able to fix atmospheric nitrogen and utilize nitrate, nitrite and ammonium for cell growth through assimilation (Park et al., 2005). Similarly, *Pseudomonas* spp. isolated from the rhizosphere of several plant species are described as diazotrophic bacteria. Nitrogen fixing pseudomonad species include *P. diminuta*, *P. fluorescens*, *P. pseudoflava*, *P. putida*, *P. saccharophila*, *P. stutzeri* and *P. vesicularis* (Chan et al., 1994). In addition, extracellular enzymes produced by *Rhodococcus* spp. are responsible for the degradation of organic compounds such as amino acids, amines and/or nitriles (Foster et al., 2014). Therefore, microbial degradation of these

nitrogen compounds may contribute to the transformation of organic matter in the soil and plant nutrition.

Similar to nitrogen, sulfur is also an important nutrient for crop plant nutrition as it is involved in cell electron transport, molecular structure and metabolic pathways regulation. Sulfur deficiency may be detected in crops grown in soil with low sulfur content, which can reduce seed yield and quality (Malhi and Gill, 2002). Among other agricultural crops grown in Western Canada, canola has a higher sulphur requirement due to its high protein content with a high proportion of the amino acids as cysteine and methionine (Malhi et al., 2005). In this study, the inoculation of canola with bacterial strains WCB1_10 (*Rhodococcus cerastrii*), and WM1_7 (*Leifsonia xyli*) increased the sulfur content in shoot, whereas strain CM3_1 (*Stenotrophomonas maltophilia*) produced an increase in the sulfur content in the shoots of wheat (Fig. 5.9). Previous studies revealed that the bacteria analyzed in the current study are involved in the sulfur cycling. For example, strains of *Rhodococcus* spp. were able to desulfonate arylsulfonates in wheat rhizospheres from long-term field wheat experiment (Schmalenberger et al., 2009). Arylsulfonates are organosulfur compounds present in soil and constitutes an important source of available sulfur for plants (Schmalenberger et al., 2009). In addition, Banerjee (2009) isolated a sulfur oxidizing *Stenotrophomonas maltophilia*, which can oxidize elemental sulfur, providing sulfate for the plants. Furthermore, sulfur oxidizing *Leifsonia* spp. also have been isolated from rhizosphere soil associated with crops (Anandham et al., 2008; Madhaiyan et al., 2010).

Results revealed that most bacterial inoculants had no significant effect on plant growth of canola and wheat grown in soil B, whereas bacterial inoculation promoted shoot biomass in these crops in soil A (Figs. 5.5 to 5.9). The agricultural soils used to grow wheat and canola exhibited differences in the organic matter content (Table 5.1). A previous study by Cakmakci et al. (2006) reported that plant growth promoting ability of bacterial inoculants varied between soils that differed in their soil organic matter content. Organic compounds in the soil may be used as carbon and energy sources by microorganisms, thus modulating microbial growth and activity (Cakmakci et al., 2006). Organic matter content also may influence the microbial community structures in the soils, and therefore, the interaction between inoculated bacteria and native soil microorganisms (Clegg et al., 2003; Marschner et al., 2003). In the current study, the two agricultural soils also differed in their concentration of available nitrogen (Table 5.1). Nitrogen is a limiting nutrient for the growth of plants and microorganisms. In soils with nutrient limitations, mutualistics

associations between rhizosphere microbial populations and plants may play important functions for plant growth. For example, rhizophere bacteria may mineralize nitrogen from soil organic matter making it available for root uptake and plant assimilation (Kuzyakov and Xu, 2013). Additionally, under limiting conditions bacteria associated with crops also may have the potential to improve plant growth and health through physiological mechanisms such as biological nitrogen fixation, phosphate solubilization, production of plant growth regulators, ACC deaminase and antibiotics (Van Elsas et al., 2006).

5.7. Conclusions

This study assessed the potential of bacterial endophytes to promote growth of canola, wheat, lentil and field pea. Several endophytic bacterial strains increased seed germination, whereas some bacteria inhibited seed germination. Canola seeds responded more favorably to inoculation, whereas detrimental effects were more frequently detected in lentil or pea plants. A total of nine bacterial strains promoted root elongation and most of these isolates also produced ACC deaminase. Inoculation of canola with strains WCB1_23 (*Agrococcus carbonis*), WCB2_14 (*Stenotrophomonas rhizophila*), WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*) and WK1_6 (*Xanthomonas fuscans*) promoted shoot growth in plants grown in a potted agricultural soil. Inoculation of canola with some of these strains also increased the nitrogen and sulfur content in shoot. Bacterial strain CM3_1 (*Stenotrophomonas maltophilia*) did not stimulate shoot growth, but increased nitrogen and sulfur content in wheat. Bacterial endophytes exhibiting promoting effects in canola growth represent a promising tool for the development of commercial inoculants that may be applied extensively in agricultural fields. However, this study revealed that soil properties may influence the effect of bacterial inoculation in crops. Therefore, field studies in different soil zones must be performed in order to assess the plant promoting effect of these bacterial strains in agroecosystems.

6. SYNTHESIS AND CONCLUSIONS

The plant microbiome includes all microbial communities colonizing the rhizosphere and plant tissues such as roots, shoots, leaves, flowers, and seeds (Orozco-Mosqueda et al., 2018). The rhizosphere microbiome has been investigated for more than 100 years (Philippot et al., 2013), but it is only recently the entire plant microbiome has come under scrutiny (Vorholt, 2012; Bulgarelli et al., 2013; Hardoim et al., 2015). Plant microbiomes consist of taxonomically diverse communities of microorganisms, including many species yet to be cultured (Müller et al., 2016). Recently, the use of high-throughput DNA sequencing techniques has provided a better understanding of the phylogenetic diversity of plant microbiomes and the functional role of microorganisms within host plants (Bulgarelli et al., 2013). Among plant associated microorganisms, bacteria colonizing plant tissues of both below- and aboveground plant organs are reported to exhibit important functions supportive of crop growth and may represent potential advantages for the establishment and development of agricultural ecosystems (Van Elsas et al., 2006).

The overall goal of this research was to investigate bacterial communities associated with canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown in agricultural fields in Saskatchewan, and to determine their potential use for plant growth promotion. Bacterial communities associated with the rhizosphere and root interior of the studied crops were assessed using high-throughput sequencing and DGGE analyses of 16S rRNA amplicons, as well as PLFA and culture dependent methods (Chapter 3). Based on the bacterial surveys reported in Chapter 3, canola and wheat were selected as test plant to investigate the bacterial microbiome associated with the rhizosphere, roots, stems, leaf and seeds at different plant growth stages (Chapter 4). Bacterial communities were assessed at stem elongation, flowering and ripening stages of canola and wheat grown in Brown and Black Chernozem soils under growth chamber conditions. Selected culturable endophytic bacteria isolated from the crops grown in agricultural fields discussed in Chapter 3, were assessed for plant growth promotion capacity (Chapter 5). The effect of candidate bacterial endophytes on seed

germination, root elongation, plant growth and nutrient uptake was determined. Production of ACC deaminase by bacterial endophytes that exhibited promoting effect on seed germination and root elongation was also assessed.

6.1. Summary of Findings

Agricultural production is an important economic activity in Saskatchewan, mainly contributing to the export of grains, oilseeds and pulses (Statistics Canada, 2017). This study provides insights on the diversity of the bacterial microbiome associated with these important crops. In chapter 3, rhizosphere and root endophytic bacterial communities were assessed in canola, wheat, field pea and lentil, growing at four agricultural fields in Saskatchewan. Subsequently, bacterial microbiomes associated with the rhizosphere, root, stem, leaf and seed of wheat and canola were assessed under controlled conditions in a growth chamber study (Chapter 4). High-throughput sequencing and DGGE analyses of 16S rRNA amplicons from bacterial communities suggested a selection of the root endophytic microbiome from the rhizosphere by crops. Previous studies also indicated that crops may be important in selecting the root microbiome, and the colonization of the root interior is not a passive process (Germida et al. 1998; Bulgarelli et al., 2013; Edwards et al. 2015). Bacterial DGGE and high-throughput sequencing profiles detected in the stems, leaves and seeds of wheat and canola varied greatly among crops, soils and plant compartments (Chapter 4). The high variability in the bacterial profiles in the aboveground plant organs may be related to the diverse habitats colonized by bacteria in the aerial parts of the plants (Compant et al., 2010). Previous studies indicated that bacterial communities associated with aboveground plant organs are influenced by several abiotic factors, which fluctuate more in the aboveground plant organs compared to the rhizosphere and root environments (Hirano and Upper, 2000).

Analysis of bacterial community structure associated with the studied four crops, indicated that soil characteristics influenced rhizosphere communities (Chapter 3 and 4). Relative abundance of Firmicutes, *Bradyrhizobium* and *Gaiella*, in the rhizosphere, as well as bacterial PLFA in the bulk soil, were significantly correlated with soil pH, silt and organic matter contents (Chapter 3). There was, however, no correlation between soil properties and the most abundant endophytic bacterial genera, thus suggesting that soil characteristics may not influence bacterial communities within the plant roots (Chapter 3). Similarly, rhizosphere bacterial communities associated with

wheat and canola grown in potted Brown and Black Chernozem soils were mainly influenced by soil characteristics (Chapter 4). As indicated by previous studies, soil properties such as pH, soil texture and soil organic contents are known to influence bacterial communities, not only in the bulk soil, but also microbial communities in the plant's rhizosphere (Van Elsas et al., 2006; Lauber et al. 2009; Carson et al., 2010).

In contrast to the rhizosphere microbiome, bacterial endophytes associated with the root interior of the studied crops were mainly influenced by the host plant, as indicated by DGGE and 16S rRNA high-throughput sequencing (Chapter 3 and 4). Gaiero et al. (2013) reported that plant factors such as differences in root morphology, composition of root exudates or the presence of wounds that may favor the penetration of bacteria into the host plant roots may influence the selection of specific bacterial endophytes. Current results also revealed that plant growth stages influenced the bacterial microbiome associated with the rhizosphere, root and aboveground plant organs of wheat and canola (Chapter 4). In addition, the analysis of the relative abundance of bacteria families associated with wheat and canola suggested that the influence of plant growth stages on the bacterial microbiome was crop and organ specific (Chapter 4). These results suggest that each crop may select specific bacterial taxa at each plant growth stage and within the different plant compartments. Previous studies also concluded that the presence of certain bacterial groups at specific growth stages was related to the different ecological strategies within rhizosphere and plant bacterial communities and their interaction with the host crop (Chiarini et al., 1998; Brimecombe et al., 2000).

Differences in bacterial communities associated with canola, wheat, field pea and lentil were detected at phylum level, since Proteobacteria, Actinobacteria and Bacteroidetes were the dominant phyla in the root interior, whereas Gemmatimonadetes and Firmicutes were only present in rhizosphere soil (Chapter 3 and 4). Additionally, Proteobacteria dominated the bacterial profiles of the aboveground plant organs of wheat and canola (Chapter 4). Proteobacteria are fast-growing microorganisms with high efficiency in metabolizing carbon substrates, thus conferring beneficial attributes that may contribute to their success in colonizing not only the rhizosphere, but also the root and aboveground plant organs (Peifer et al., 2013; Fierer et al., 2007; García-Salamanca et al., 2012).

Analysis of bacterial communities at genera level, indicated that *Pseudomonas* and *Stenotrophomonas* were predominant genera in the rhizosphere and root interior of all crops grown in Saskatchewan agricultural soils, thus suggesting a generalist distribution of these bacteria (Chapter 3). Bacterial endophytes considered as generalists may potentially produce beneficial effects on numerous plant species (Compant et al., 2005). Additional bacterial genera were predominant only in certain crops, suggesting that selection of bacterial consortia associated with the root interior of canola, wheat, pea and lentil was crop-specific (Chapter 3 and 4). As expected, *Rhizobium* was the most dominant genus in pea and lentil grown in agricultural fields in Saskatchewan (Chapter 3). When the high-throughput sequencing data were combined from all the studies assessing the bacterial communities associated with canola and wheat grown in Brown and Black Chernozem soils, differences were detected in the endophytic bacteria communities depending of the experimental conditions (Figs. 6.1 and 6.2). For example, canola roots from the growth chamber (Fig. 6.1, Cluster A) experiment were characterized by a dominance of *Citrobacter* (31%) and unclassified members of Enterobacteriaceae (5%) and Pseudonocardiaceae (12%) (Chapter 4). In contrast, endophytic communities associated with canola roots collected in agricultural fields (Fig. 6.1, Cluster B) exhibited a high abundance of *Pseudomonas* (30%), *Stenotrophomonas* (11%), *Citrobacter* (8%), *Acinetobacter* (8%) as well as unclassified members of Enterobacteriaceae (8%) (Chapter 3). Similarly, endophytic communities of wheat collected in agricultural fields (Fig. 6.2, Clusters A and C) were dominated by *Pseudomonas* (22%) *Streptomyces* (10%), *Xanthomonas* (5%) and unclassified members Comamonadaceae (6%) and Enterobacteriaceae (6%) (Chapter 3), whereas in growth chamber conditions (Fig. 6.2, Cluster B) a high abundance of *Pseudomonas* (6%) *Streptomyces* (6%), *Citrobacter* (5%), and Chitinophaga (5%) and unclassified members Comamonadaceae (8%) and Rhizobiaceae (8%) was detected (Chapter 4). These results suggest that endophytic bacterial communities associated with crops were influenced by experimental factors prevailing in agricultural fields or growth chamber conditions. Furthermore, rhizosphere bacteria profiles of wheat and canola also varied between plants collected at agriculture fields (Chapter 3) and growth chamber experiments (Chapter 4). However, bacterial communities associated with the rhizosphere of these crops grown in the field or growth chamber conditions were mainly modulated by soil characteristics (Figs. 6.3 and 6.4). Bacterial communities associated with the aboveground plant organs were enriched with *Pseudomonas* and unclassified members of Enterobacteriaceae (Chapter 4). Furthermore, in both

canola and wheat several bacterial genera were only detected in the aboveground plant organs suggesting that these bacteria originated initially in the phyllosphere of the plant and unlikely in the root and/or soil (Chapter 4).

Bacterial endophyte strains (n=298) were isolated from the roots of canola, wheat, pea and lentil using cultivation methods (Chapter 3). Most commonly found endophytic bacteria associated to the crops included *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Stenotrophomonas*. A total of 157 bacterial endophytes were selected and assessed for their effect on plant growth (Chapter 5). Several endophytic strains produced an increased seed germination rate, whereas some strains inhibited seed germination. Canola seeds responded more favorable to inoculation, whereas detrimental effect was detected more frequently in lentil or pea plants. Root elongation assay and ACC deaminase were assessed on the 40 isolates that exhibited the highest germination rates on the host crops. Stimulation of root elongation was produced by nine bacterial endophytes in canola and wheat. Previous studies reported that the production of ACC deaminase is a key mechanism involved in the promotion of plant growth (Glick, 2014). However, in the current study, ACC deaminase activity was detected in 16 bacterial strains, from which only six strains increased root elongation. These results suggest that multiple bacterial mechanisms may be involved on the stimulation of root elongation of the four studied crops (Chapter 5).

Candidate bacterial strains that exhibited promoting effects on seed germination and root elongation were tested for plant growth promotion in wheat and canola grown in two agricultural soils collected in Central Butte, Saskatchewan, Canada. Inoculation with strains WCB1_23 (*Agrococcus carbonis*), WCB2_14 (*Stenotrophomonas rhizophila*), WM1_7 (*Leifsonia xyli*), CS1_1 (*Pantoea vagans*) and WK1_6 (*Xanthomonas fuscans*) promoted shoot growth in canola grown in potted soils. Some of these strains also produced an increase in the nitrogen and sulfur content of canola. Based on their promoting effect on canola growth, these endophytes may represent a promising biological tool for the development of commercial inoculants that may be applied extensively in agricultural fields. Bacterial strain CM3_1 (*Stenotrophomonas maltophilia*) did not stimulate shoot growth in wheat but increased the concentration of available nitrogen and sulfur in the shoots. Most bacterial inoculants did not significantly affect plant growth of canola or wheat grown in low organic matter soil, whereas bacterial inoculation in soil with a higher organic matter content promoted shoot biomass in these crops (Chapter 5). These results suggest that soil properties may have influenced the effect of bacterial inoculation in crops.

6.2. Future Research

The research presented in this dissertation contributes to the current knowledge on plant bacterial microbiomes. Emphasis was placed on bacteria that exhibited potential for plant growth promotion in economically important crops. Understanding the interactions and dynamic of plant microbiomes requires the assessment of bacterial communities present in different plant organs at various plant development stages. Due to the importance of root associated bacteria for plant growth, this study investigated bacterial communities associated with the rhizosphere and root interior of canola, wheat, field pea and lentil grown in agricultural fields in Saskatchewan. Results demonstrated that crops may select root endophytic communities, suggesting that future studies also may focus on the endophytic microbiome associated with different crop cultivars as well as in crop breeding studies. Additional research investigating bacterial communities associated with stem, leaf and seeds of commercial crops may focus on crops grown in the field. Moreover, future studies are needed to assess the effects of biotic and abiotic factors on bacterial communities associated with aboveground plant organs. In addition, to have a better understanding of bacteria distribution and function in agricultural ecosystems, the influence of soil physical and chemical factors on growth of individual bacterial species inhabiting the rhizosphere of crops must be investigated.

In the current study, several endophytic bacteria isolated from roots demonstrated promising plant growth promotion effects in wheat and canola in a growth chamber experiment. Future field studies may be conducted to confirm the results observed in the laboratory and growth chamber experiments. For these experiments, survival of inoculants as well as the effect of inoculation on the native plant associated microbiome should be assessed in plants grown in field trials. Testing the effect of endophytic bacteria isolated from seeds also may provide new alternatives for development of seed inoculants that can be used for commercial crops. The use of metagenomics tools may be an important asset when assessing the influence of bacterial inoculation on the expression of genes involved in plant growth and yield.

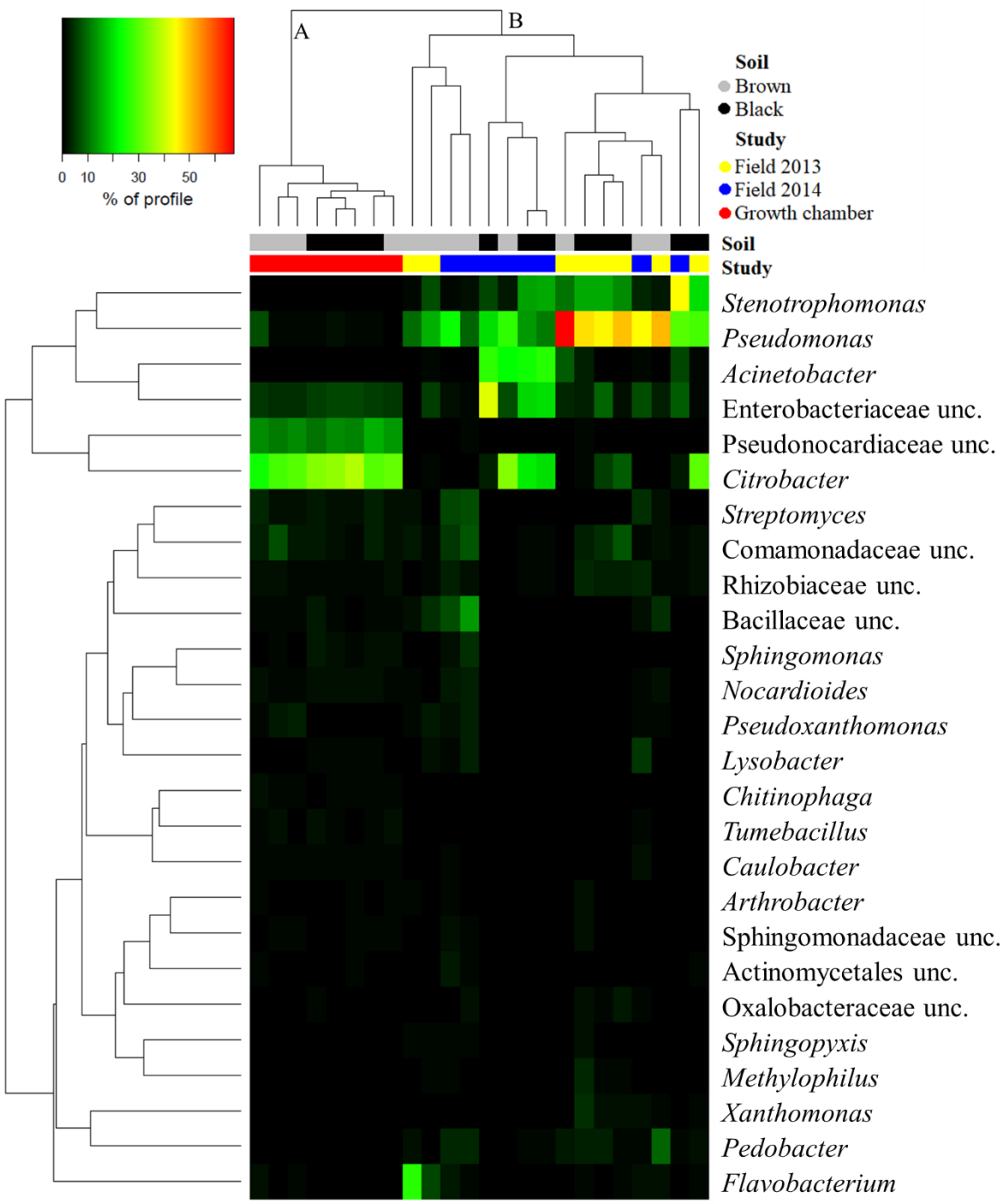

Fig. 6.1. Hierarchical clustering (Bray-Curtis) of bacterial genera (>0.5% abundant) associated with the root interior of canola grown in field conditions and growth chamber on Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

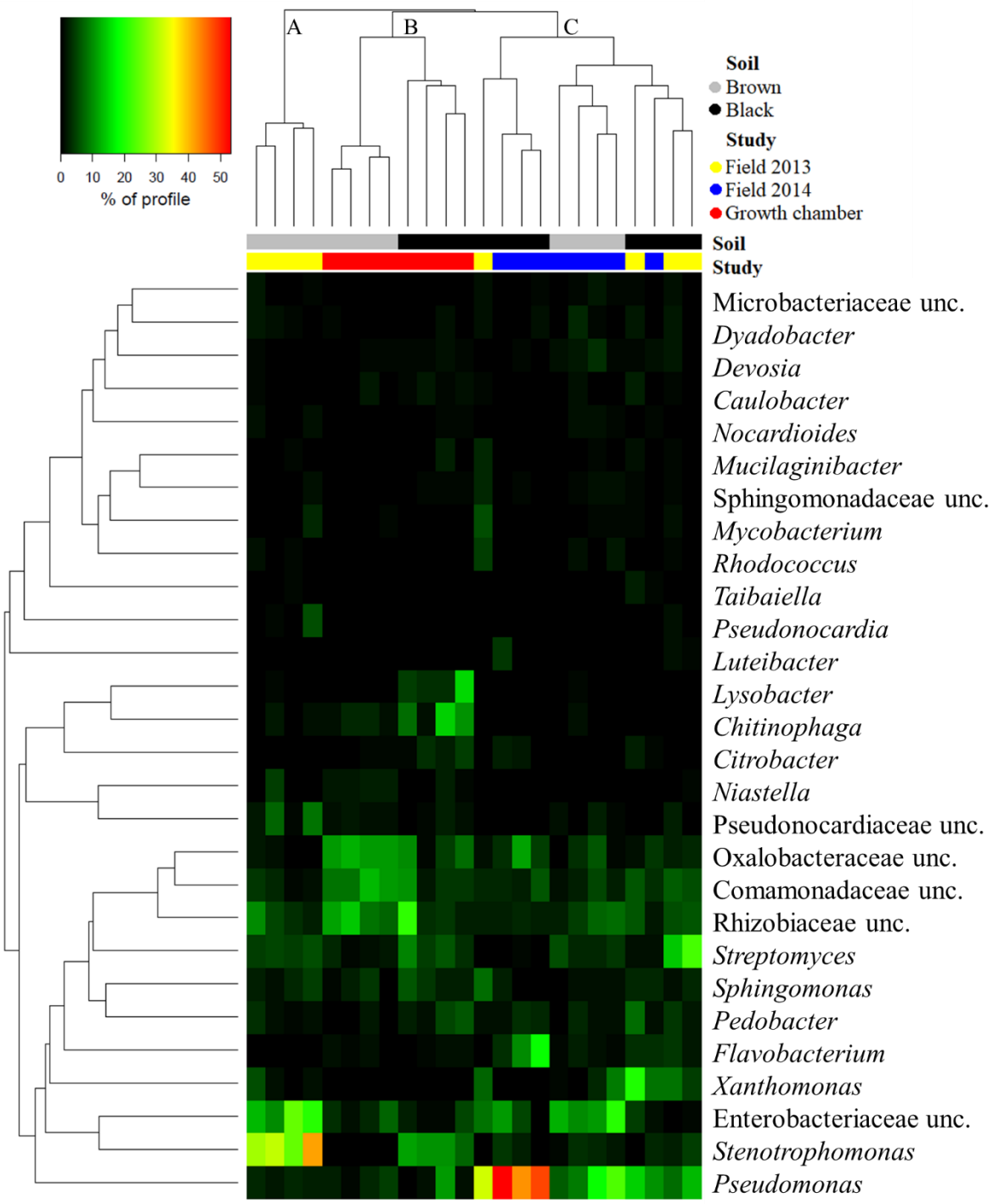

Fig. 6.2. Hierarchical clustering (Bray-Curtis) of bacterial genera (>0.5% abundant) associated with the root interior of wheat grown in field conditions and growth chamber on Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

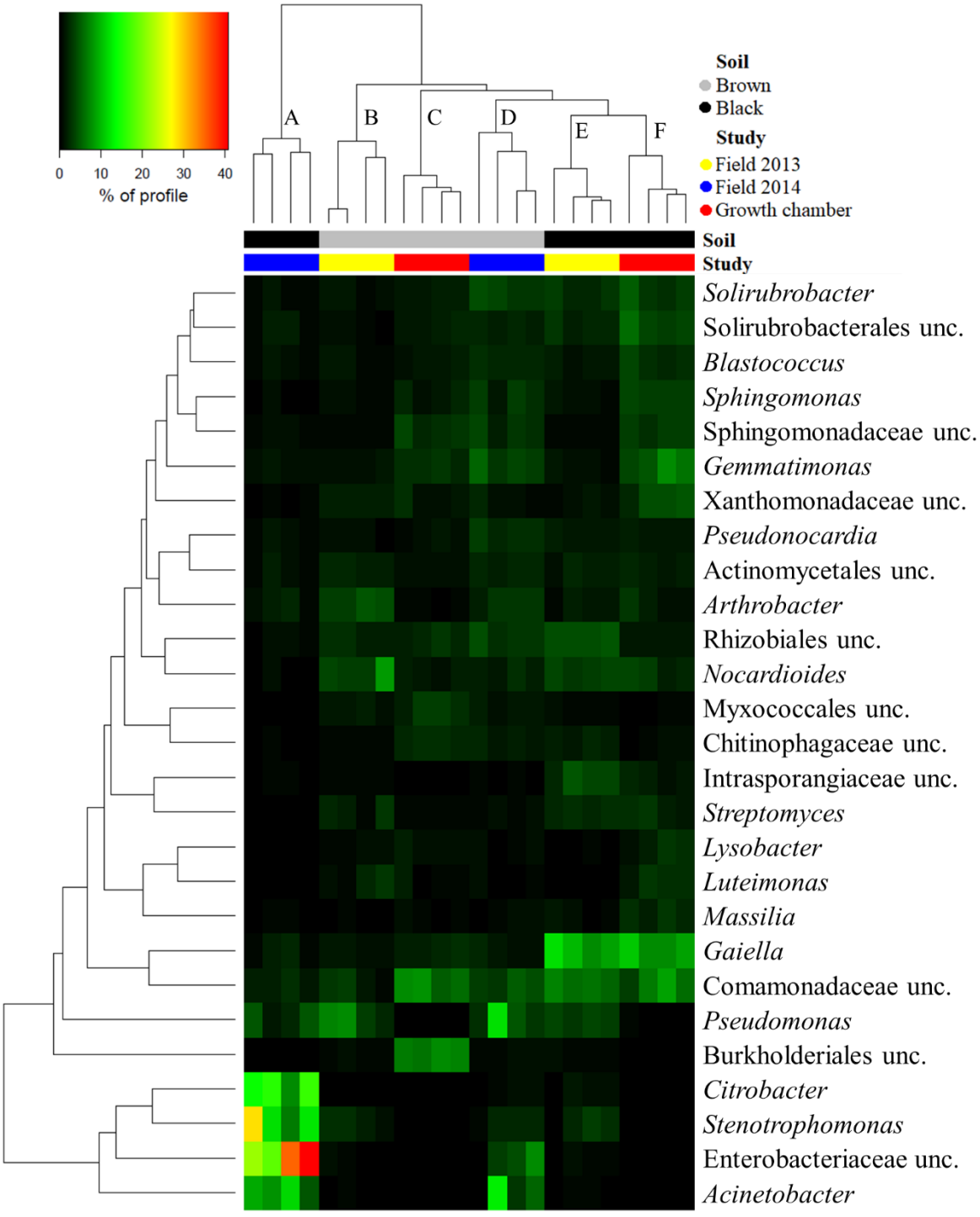

Fig. 6.3. Hierarchical clustering (Bray-Curtis) of bacterial genera (>1% abundant) associated with the rhizosphere of canola grown in field conditions and growth chamber on Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

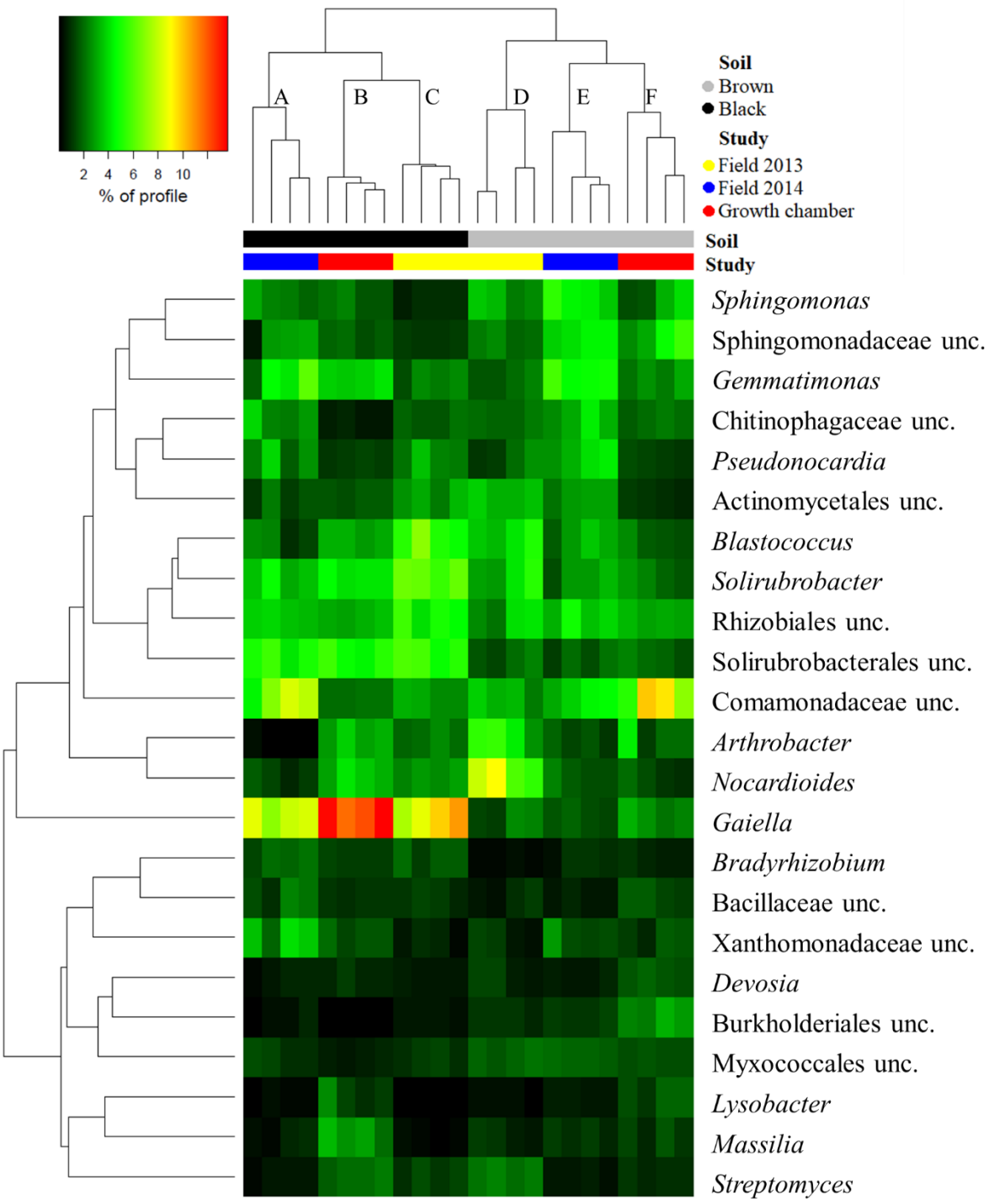

Fig. 6.4. Hierarchical clustering (Bray-Curtis) of bacterial genera (>1% abundant) associated with the rhizosphere of wheat grown in field conditions and growth chamber on Brown and Black agricultural soils from Central Butte and Melfort, Saskatchewan, respectively.

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APPENDIX A: Location of agricultural field sites sampled in Saskatchewan and sampling strategy

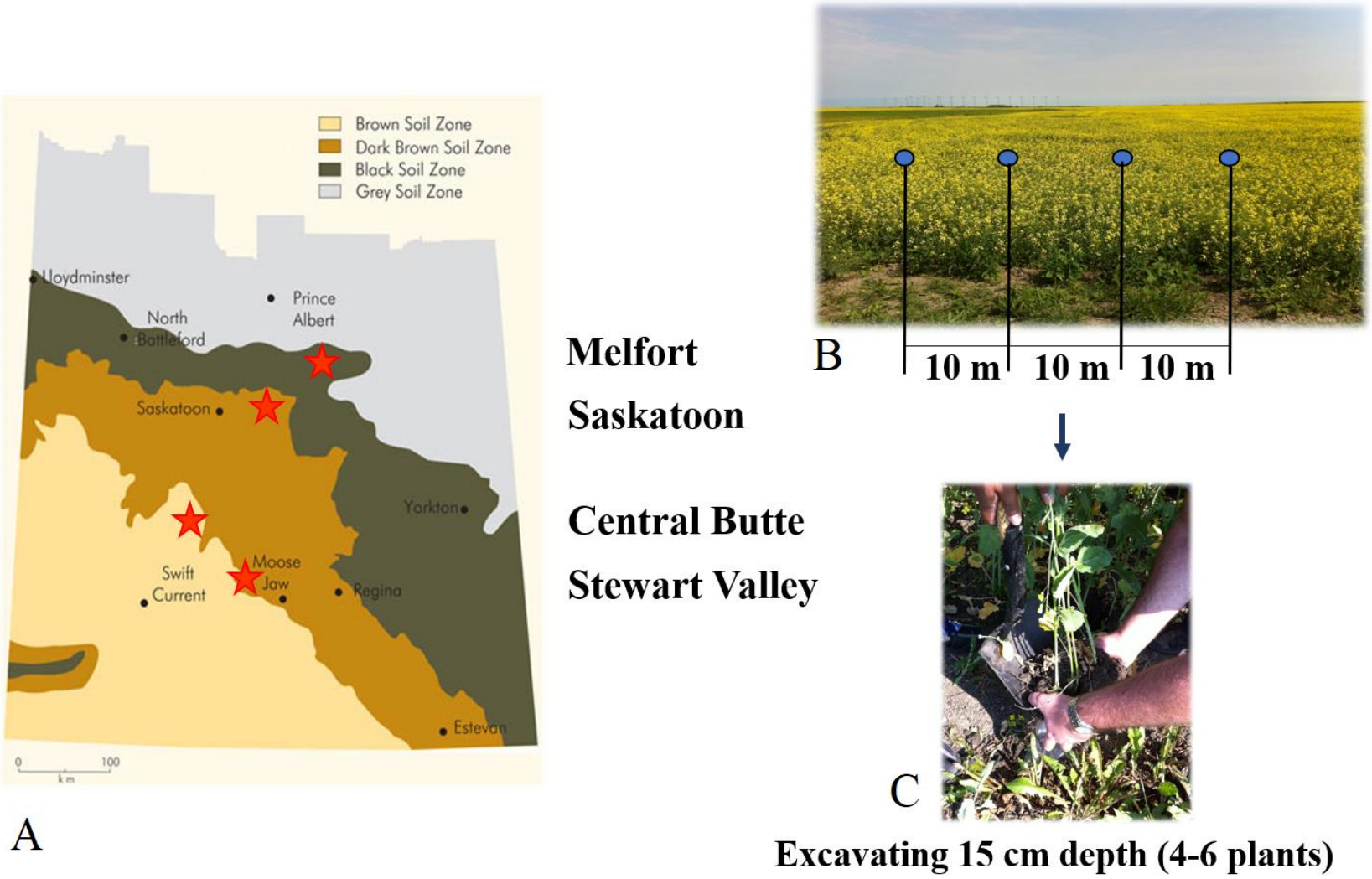

Fig. A.1. Location of agricultural field sites sampled in Saskatchewan during 2013 and 2014 (A), sampling points (B) and sampling procedure (C).

APPENDIX B: Identification of endophytic bacteria associated with canola, wheat, field pea and lentil grown at agricultural soils from Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan

Table B.1. Identification of culturable endophytic bacteria associated with canola, wheat, pea and lentil grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

ID	Crop	Location	Closest match	Similarity (%)
C1	canola	CB	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
C2	canola	CB	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
C3	canola	CB	*Brucella ceti* 16SrRNA	96
C4	canola	CB	*Streptomyces tacrolimicus* strain ATCC 55098 16SrRNA	98
C5	canola	CB	*Arthrobacter phenanthrenivorans* strain Sphe3 16SrRNA	99
C6	canola	CB	*Serratia liquefaciens* strain ATCC 27592 16SrRNA	98
C7	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	94
C8	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	95
C9	canola	CB	*Methylophilus quaylei* strain M 16SrRNA	88
C10	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	98
C11	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	94
C12	canola	CB	*Stenotrophomonas maltophilia* R551-3 strain R551-3 16SrRNA	79
C13	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	78
C14	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	98
C15	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	92
C16	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	94
C17	canola	CB	*Streptomyces tacrolimicus* strain ATCC 55098 16SrRNA	97
C18	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	98
C19	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	98
C20	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	98
C21	canola	CB	*Stenotrophomonas maltophilia* strain ATCC 19861 16SrRNA	96
C22	canola	CB	*Brucella ceti* 16SrRNA	97
C23	canola	CB	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
C24	canola	CB	*Microbacterium murale* strain 01-Gi-001 16SrRNA	97
C25	canola	SV	*Pantoea vagans* C9-1 strain C9-1 16SrRNA	99
C26	canola	SV	*Pantoea vagans* C9-1 strain C9-1 16SrRNA	99
C27	canola	SV	*Pantoea vagans* C9-1 strain C9-1 16SrRNA	100
C28	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16SrRNA	99
C29	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16SrRNA	96
C30	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16SrRNA	98
C31	canola	SV	*Bacillus aryabhattai* strain B8W22 16SrRNA	99
C32	canola	SV	*Bacillus aryabhattai* strain B8W22 16SrRNA	99

ID	Crop	Location	Closest match	Similarity (%)
C33	canola	SV	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
C34	canola	SV	*Bacillus abyssalis* strain SCSIO 15042 16S rRNA	95
C35	canola	SV	*Bacillus aryabhattai* strain B8W22 16S rRNA	97
C36	canola	SV	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
C37	canola	SV	*Pseudomonas mediterranea* strain CFBP 5447 16S rRNA	98
C38	canola	SV	*Pseudomonas mediterranea* strain CFBP 5447 16S rRNA	98
C39	canola	SV	*Pseudomonas poae* RE*1-1-14 strain RE*1-1-14 16S rRNA	98
C40	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16S rRNA	86
C41	canola	SV	*Xanthomonas fuscans* subsp. *fuscans* 16S rRNA	92
C42	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16S rRNA	90
C43	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16S rRNA	95
C44	canola	SV	*Acinetobacter calcoaceticus* strain ATCC 23055 16S rRNA	98
C45	canola	SN	*Microbacterium testaceum* StLB037 strain StLB037 16S rRNA	98
C46	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C47	canola	SN	*Microbacterium testaceum* StLB037 strain StLB037 16S rRNA	99
C48	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C49	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	98
C50	canola	SN	*Streptomyces nigrescens* strain NRRL B-12176 16S rRNA	85
C51	canola	SN	*Pseudomonas tolaasii* strain ATCC 33618 16S rRNA	97
C52	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C53	canola	SN	*Pseudomonas tolaasii* strain ATCC 33618 16S rRNA	97
C54	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C55	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C56	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	98
C57	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C58	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C59	canola	SN	*Enterococcus saccharolyticus* strain LMG 11427 16S rRNA	85
C60	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	96
C61	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C62	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C63	canola	SN	*Streptomyces tacrolimicus* strain ATCC 55098 16S rRNA	99
C64	canola	MF	*Stenotrophomonas maltophilia* R551-3 strain R551-3 16S rRNA	96
C65	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C66	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	98
C67	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	93

ID	Crop	Location	Closest match	Similarity (%)
C68	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	95
C69	canola	MF	*Stenotrophomonas* sp. ICB194 16S rRNA	84
C70	canola	MF	*Stenotrophomonas maltophilia* R551-3 strain R551-3 16S rRNA	94
C71	canola	MF	*Pseudoxanthomonas koreensis* strain NBRC 101160 16S rRNA	87
C72	canola	MF	*Pseudomonas monteilii* 16S rRNA	88
C73	canola	MF	*Bacillus halosaccharovorans* strain E33 16S rRNA	79
C74	canola	MF	*Stenotrophomonas pavanii* strain ICB 89 16S rRNA	91
C75	canola	MF	*Neisseria flava* strain NRL 30008 16S rRNA	89
C76	canola	MF	*Pseudoxanthomonas taiwanensis* strain NBRC 101072 16S rRNA	83
C77	canola	MF	*Mesorhizobium australicum* strain WSM2073 16S rRNA	97
C78	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C79	canola	MF	*Stenotrophomonas maltophilia* R551-3 strain R551-3 16S rRNA	95
C80	canola	MF	*Chryseobacterium humi* strain ECP37 16S rRNA	82
C81	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C82	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	97
C83	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	98
C84	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C85	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	88
C86	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	94
C87	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C88	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	97
C89	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
C90	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	97
C91	canola	MF	*Stenotrophomonas maltophilia* strain ATCC 19861 16S rRNA	96
W1	wheat	CB	*Brevibacillus agri* strain X6PO3 16S rRNA	93
W2	wheat	CB	*Brevibacillus agri* strain DM8-3 16S rRNA	80
W3	wheat	CB	*Brevibacillus agri* strain USH5 16S rRNA	93
W4	wheat	CB	*Nitrosospira multiformis* strain ATCC 25196 16S rRNA	83
W5	wheat	CB	*Brevibacillus agri* strain NBRC 15538 16S rRNA	95
W6	wheat	CB	*Agrococcus citreus* strain DSM 12453 16S rRNA	97
W7	wheat	CB	*Kinneretia asaccharophila* strain KIN192 16S rRNA	96
W8	wheat	CB	*Veillonellaceae* bacterium oral taxon 155 clone VU007 16S rRNA	96
W9	wheat	CB	*Rhizobium* sp. Q54 16S rRNA	95
W10	wheat	CB	*Rhodococcus cerastrii* strain C5 16S rRNA	98
W11	wheat	CB	*Rhodococcus cercidiphylli* strain YIM 65003 16S rRNA	99

ID	Crop	Location	Closest match	Similarity (%)
W12	wheat	CB	*Rhodococcus* sp. RS-75 16SrRNA	99
W13	wheat	CB	*Arthrobacter subterraneus* strain Tibetlhz-13 16SrRNA	97
W14	wheat	CB	*Mycetocola manganoxydans* strain BKKb2 16SrRNA	97
W15	wheat	CB	*Stenotrophomonas rhizophila* strain e-p10 16SrRNA	98
W16	wheat	CB	*Rhizobium skierniewicense* strain CH11 16SrRNA	99
W17	wheat	CB	*Williamsia marianensis* strain DSM 44944 16SrRNA	100
W18	wheat	CB	*Streptomyces tacrolimicus* strain ATCC 55098 16SrRNA	98
W19	wheat	CB	*Williamsia marianensis* strain DSM 44944 16SrRNA	100
W20	wheat	CB	*Streptomyces griseus* subsp. *rhodochrous* strain Rsh04-07 16SrRNA	97
W21	wheat	CB	*Williamsia marianensis* strain DSM 44944 16SrRNA	90
W22	wheat	CB	*Agrococcus carbonis* strain G4 16SrRNA	95
W23	wheat	CB	*Streptomyces griseus* subsp. *rhodochrous* strain Rsh04-07 16SrRNA	97
W24	wheat	SN	*Paenibacillus taohuashanense* strain gs65 16SrRNA	99
W25	wheat	SN	*Paenibacillus taohuashanense* strain gs65 16SrRNA	97
W26	wheat	SN	*Microbacterium* sp. SO3-3N 16SrRNA	99
W27	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	99
W28	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	98
W29	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	99
W30	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	96
W31	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	99
W32	wheat	SN	*Xanthomonas fuscans* subsp. *fuscans* 16SrRNA	99
W33	wheat	SN	*Stenotrophomonas pavanii* strain LMG 25348 16SrRNA	98
W34	wheat	SN	*Xanthomonas campestris* strain ATCC 33913 16SrRNA	99
W35	wheat	SN	*Microbacterium* sp. SO3-3N 16SrRNA	99
W36	wheat	SN	*Mycetocola zhadangensis* strain ZD1-4 16SrRNA	97
W37	wheat	SN	*Mycetocola zhadangensis* strain ZD1-4 16SrRNA	99
W38	wheat	SN	*Paenibacillus naphthalenovorans* strain PR-N1 16SrRNA	99
W39	wheat	SN	*Pseudomonas fluorescens* Pf0-1 strain Pf0-1 16SrRNA	97
W40	wheat	SN	*Pseudomonas fluorescens* Pf0-1 strain Pf0-1 16SrRNA	96
W41	wheat	SN	*Pseudomonas fluorescens* Pf0-1 strain Pf0-1 16SrRNA	93
W42	wheat	SN	*Pseudomonas fluorescens* Pf0-1 strain Pf0-1 16SrRNA	98
W43	wheat	SN	*Brevibacillus agri* strain NBRC 15538 16SrRNA	97
W44	wheat	MF	*Rhizobium skierniewicense* strain CH11 16SrRNA	99
W45	wheat	MF	*Brevibacillus agri* strain NBRC 15538 16SrRNA	89
W46	wheat	MF	*Luteimonas huabeiensis* strain HB2 16SrRNA	92

ID	Crop	Location	Closest match	Similarity (%)
W47	wheat	MF	*Stenotrophomonas rhizophila* strain e-p10 16S rRNA	92
W48	wheat	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	99
W49	wheat	MF	*Pseudomonas fluorescens* Pf0-1 strain Pf0-1 16S rRNA	92
W50	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	98
W51	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	99
W52	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	96
W53	wheat	MF	*Plantibacter aurantiacus* partial 16S rRNA	96
W54	wheat	MF	*Microbacterium saccharophilum* strain K-1 16S rRNA	98
W55	wheat	MF	*Microbacterium saccharophilum* strain K-1 16S rRNA	97
W56	wheat	MF	*Mesorhizobium australicum* strain WSM2073 16S rRNA	98
W57	wheat	MF	*Galbitalea soli* strain KIS82-1 16S rRNA	97
W58	wheat	MF	*Galbitalea soli* strain KIS82-1 16S rRNA	99
W59	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	100
W61	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	100
W62	wheat	MF	*Mycobacterium smegmatis* strain DSM 43756 16S rRNA	100
W63	wheat	MF	*Rhizobium skierniewicense* strain CH11 16S rRNA	96
W64	wheat	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	97
W65	wheat	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	100
W66	wheat	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	97
W67	wheat	MF	*Leifsonia xyli* subsp. *xyli* str. CTCB07 strain CTCB07 16S rRNA	96
L1	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16S rRNA	99
L2	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	99
L3	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	99
L4	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	99
L5	lentil	CB	*Bordetella hinzii* strain LMG 13501 16S rRNA	96
L6	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	97
L7	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	98
L8	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	97
L9	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	99
L10	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	97
L11	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16S rRNA	98
L12	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16S rRNA	98
L13	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16S rRNA	96
L14	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	96
L15	lentil	CB	*Pantoea agglomerans* strain DSM 3493 16S rRNA	97

ID	Crop	Location	Closest match	Similarity (%)
L16	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16SrRNA	97
L17	lentil	CB	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421 16SrRNA	96
L18	lentil	CB	*Pseudomonas tolaasii* strain ATCC 33618 16SrRNA	97
L19	lentil	SV	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	95
L20	lentil	SV	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	99
L21	lentil	SV	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	99
L22	lentil	SV	*Pantoea agglomerans* strain DSM 3493 16SrRNA	95
L23	lentil	SV	*Pantoea agglomerans* strain DSM 3493 16SrRNA	99
L24	lentil	SV	*Pantoea agglomerans* strain DSM 3493 16SrRNA	99
L25	lentil	SV	*Pantoea agglomerans* strain DSM 3493 16SrRNA	93
L26	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
L27	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	98
L28	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
L29	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	98
L30	lentil	SV	*Methylobacterium populi* strain BJ001 16SrRNA	86
L31	lentil	SV	*Rhodococcus phenolicus* strain DSM 44812 16SrRNA	97
L32	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
L33	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
L34	lentil	SV	*Microbacterium mangrovi* strain MUSC 115 16SrRNA	96
L35	lentil	SV	*Microbacterium murale* strain 01-Gi-001 16SrRNA	99
L36	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	75
L37	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	88
L38	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	97
L39	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	98
L40	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	97
L41	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	99
L42	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	97
L43	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	99
L44	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	99
L45	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	99
L46	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	99
L47	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	95
L48	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	99
L49	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16SrRNA	99
L50	lentil	SN	*Bacillus halosaccharovorans* strain E33 16SrRNA	98

ID	Crop	Location	Closest match	Similarity (%)
L51	lentil	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	98
L52	lentil	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
L53	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16S rRNA	98
L54	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* 3841 strain 3841 16S rRNA	99
L55	lentil	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
L56	lentil	SN	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	96
L57	lentil	SN	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	99
L58	lentil	SN	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	98
L59	lentil	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
P1	pea	CB	*Novosphingobium* sp. PP1Y strain PP1Y 16S rRNA	98
P2	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	98
P3	pea	CB	*Novosphingobium sp*. PP1Y strain PP1Y 16S rRNA	99
P4	pea	CB	*Sinorhizobium fredii* NGR234 strain NGR234 16S rRNA	95
P5	pea	CB	*Sinorhizobium fredii* NGR234 strain NGR234 16S rRNA	97
P6	pea	CB	*Rhizobium lemnae* strain L6-16 16S rRNA	98
P7	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	99
P8	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	99
P9	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	99
P10	pea	CB	*Escherichia vulneris* strain NBRC 102420 16S rRNA	96
P11	pea	CB	*Stenotrophomonas rhizophila* strain e-p10 16S rRNA	98
P12	pea	CB	*Pseudomonas mucidolens* strain NBRC 103159 16S rRNA	91
P13	pea	CB	*Pseudomonas rhodesiae* strain CIP 104664 16S rRNA	96
P14	pea	CB	*Pseudomonas mucidolens* strain NBRC 103159 16S rRNA	94
P15	pea	CB	*Stenotrophomonas rhizophila* strain e-p10 16S rRNA	98
P16	pea	CB	*Stenotrophomonas rhizophila* strain e-p10 16S rRNA	98
P17	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	99
P18	pea	CB	*Microbacterium mangrovi* strain MUSC 115 16S rRNA	98
P19	pea	CB	*Rhizobium rosettiformans* strain W3 16S rRNA	94
P20	pea	CB	*Rhizobium rosettiformans* strain W3 16S rRNA	96
P21	pea	CB	*Rhizobium lemnae* strain L6-16 16S rRNA	99
P22	pea	CB	*Microbacterium murale* strain 01-Gi-001 16S rRNA	98
P23	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	98
P24	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	98
P25	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	97
P26	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	96

ID	Crop	Location	Closest match	Similarity (%)
P27	pea	SV	*Stenotrophomonas pavanii* strain LMG 25348 16S rRNA	97
P28	pea	SV	*Microbacterium murale* strain 01-Gi-001 16S rRNA	96
P29	pea	SV	*Pantoea agglomerans* strain DSM 3493 16S rRNA	96
P30	pea	SV	*Achromobacter xylosoxidans* A8 strain A8 16S rRNA	97
P31	pea	SV	*Rhizobium lemnae* strain L6-16 16S rRNA	98
P32	pea	SV	*Bacillus vireti* strain NBRC 102452 16S rRNA	98
P33	pea	SV	*Bacillus halosaccharovorans* strain E33 16S rRNA	99
P34	pea	SV	*Bacillus benzoevorans* strain NCIMB 12555 16S rRNA	98
P35	pea	SV	*Rhizobium lemnae* strain L6-16 16S rRNA	98
P36	pea	SV	*Bacillus halosaccharovorans* strain E33 16S rRNA	98
P37	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	94
P38	pea	SV	*Bacillus halosaccharovorans* strain E33 16S rRNA	97
P39	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	98
P40	pea	SV	*Rhizobium lemnae* strain L6-16 16S rRNA	93
P41	pea	SV	*Selenomonas bovis* strain WG 16S rRNA	92
P42	pea	SV	*Selenomonas flueggei* strain ATCC 43531 16S rRNA	93
P43	pea	SV	*Paenibacillus pabuli* strain NBRC 13638 16S rRNA	95
P44	pea	SN	*Arthrobacter phenanthrenivorans* strain Sphe3 16S rRNA	97
P45	pea	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	98
P46	pea	SN	*Bacillus atrophaeus* 1942 strain 1942 16S rRNA	99
P47	pea	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	98
P48	pea	SN	*Dokdonella immobilis* strain LM 2-5 16S rRNA	92
P49	pea	SN	*Fictibacillus rigui* strain WPCB074 16S rRNA	95
P50	pea	SN	*Staphylococcus epidermidis* RP62A strain RP62A 16S rRNA	95
P51	pea	SN	*Rhizobium lemnae* strain L6-16 16S rRNA	95
P52	pea	SN	*Rhizobium lemnae* strain L6-16 16S rRNA	98
P53	pea	SN	*Bacillus halosaccharovorans* strain E33 16S rRNA	97
P54	pea	SN	*Paenibacillus naphthalenovorans* strain PR-N1 16S rRNA	93
P55	pea	SN	*Paenibacillus naphthalenovorans* strain PR-N1 16S rRNA	96
P56	pea	SN	*Paenibacillus naphthalenovorans* strain PR-N1 16S rRNA	98
P57	pea	SN	*Bacillus firmus* strain NBRC 15306 16S rRNA	99
P58	pea	SN	*Bacillus circulans* strain ATCC 4513 16S rRNA	99
P59	pea	SN	*Bacillus circulans* strain ATCC 4513 16S rRNA	99
P60	pea	SN	*Bacillus circulans* strain ATCC 4513 16S rRNA	98
P61	pea	SN	*Bacillus circulans* strain ATCC 4513 16S rRNA	99

ID	Crop	Location	Closest match	Similarity (%)
P62	pea	SN	*Bacillus circulans* strain ATCC 4513 16S rRNA	98
P63	pea	MF	*Pseudomonas* sp. CB13 16S rRNA	98
P64	pea	MF	*Rhizobium lemnae* strain L6-16 16S rRNA	97
P65	pea	MF	*Rhizobium lemnae* strain L6-16 16S rRNA	95
P66	pea	MF	*Paenibacillus ginsengisoli* strain LMG 23406 16S rRNA	97
P67	pea	MF	*Bacillus halosaccharovorans* strain E33 16S rRNA	97
P68	pea	MF	*Paenibacillus naphthalenovorans* strain PR-N1 16S rRNA	98
P69	pea	MF	*Arthrobacter phenanthrenivorans* strain Sphe3 16S rRNA	98
P70	pea	MF	*Pantoea vagans* C9-1 strain C9-1 16S rRNA	99
P71	pea	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	97
P72	pea	MF	*Pantoea vagans* C9-1 strain C9-1 16S rRNA	97
P73	pea	MF	*Erwinia tasmaniensis* strain Et1/99 16S rRNA	99
P74	pea	MF	*Pantoea agglomerans* strain DSM 3493 16S rRNA	97
P75	pea	MF	*Paenibacillus naphthalenovorans* strain PR-N1 16S rRNA	96
P76	pea	MF	*Paenibacillus naphthalenovorans strain* PR-N1 16S rRNA	95
P77	pea	MF	*Bacillus halosaccharovorans* strain E33 16S rRNA	97
P78	pea	MF	*Bacillus halosaccharovorans* strain E33 16S rRNA	98
P79	pea	MF	*Bacillus halosaccharovorans* strain E33 16S rRNA	98

Table B.2. Number of culturable endophytic bacteria isolated from the roots of canola, wheat, pea and lentil collected at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan. Genera were classified by phyla: Proteobacteria (P), Actinobacteria (A), Firmicutes (F) and Bacteroidetes (B).

	Crop	Canola				Wheat			Pea				Lentil		
	Location	CB	SV	SN	MF	CB	SN	MF	CB	SV	SN	MF	CB	SV	SN
Classification	Phylum														
Rhizobium	P					2		1	4		1	4		3	5
Sinorhizobium	P								2						
Mesorhizobium	P				1			1							
Methylobacterium	P													1	
Brucella	P	2													
Novosphingobium	P								2						
Neisseria	P				1										
Methylophilus	P	1													
Bordetella	P												1		
Achromobacter	P									1					
Kinneretia	P					1									
Nitrosospira	P					1									
Stenotrophomonas	P	13			20	1	2		3	1					
Xanthomonas	P		1				7								
Pseudoxanthomonas	P				2										
Luteimonas	P						1								
Dokdonella	P										1				
Pseudomonas	P		3	2	1		4					1	7	3	
Acinetobacter	P		7												
Pantoea	P		3							1		3	10	4	
Erwinia	P						1	4				2			
Serratia	P	1													
Escherichia	A								1						
Microbacterium	A	4		4			2	2	7	1				7	
Streptomyces	A	2		14		3									
Mycobacterium	A							6							
Arthrobacter	A	1				1					1	1			
Rhodococcus	A					3								1	
Mycetocola	A					1	2								
Williamsia	A					3									
Leifsonia	A							3							
Agrococcus	A					2									
Galbitalea	A							2							
Plantibacter	A							1							
Bacillus	F		6		1					5	10	4		4	12
Paenibacillus	F						3			10	7	2			3
Brevibacillus	F					4	2								
Fictibacillus	F										1				
Staphylococcus	F										1				
Enterococcus	F			1											
Selenomonas	F									2					
Chryseobacterium	B				1										
Total		24	20	21	27	22	24	20	19	21	22	17	18	23	20
Total/crop		92				66			79				61		

Table B.3. Phylogenetic affiliation of endophytic bacteria associated with canola, wheat, pea and lentil grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan, based on 16S rRNA sequences amplified from dominant DGGE bands.

ID	Crop	Location	Closest match	Similarity (%)
C1	canola	CB	*Pseudomonas* sp. GTYR-8 16SrRNA	89%
C2	canola	CB	*Pseudomonas fluorescens* strain B20 16SrRNA	99%
C3	canola	CB	*Pseudomonas* sp. 01xTSA06A_H02 16SrRNA	97%
C4	canola	CB	*Pseudomonas brassicacearum* strain DF41	100%
C5	canola	CB	*Pseudomonas brassicacearum* strain DF41	99%
C6	canola	CB	Uncultured bacterium clone Upland_120_2953 16SrRNA	99%
C7	canola	CB	Uncultured bacterium clone 24c12 16SrRNA	99%
C8	canola	CB	*Enterobacter cloacae* subsp. *dissolvens* strain A8 16SrRNA	99%
C9	canola	CB	*Stenotrophomonas maltophilia* strain BU100 16SrRNA	86%
C10	canola	CB	*Stenotrophomonas* sp. Artichoke A1 16SrRNA	98%
C11	canola	CB	*Enterobacter lignolyticus* SCF1	83%
C12	canola	CB	*Burkholderia* sp. III-116a-32 16SrRNA	99%
C13	canola	CB	*Burkholderia* sp. III-116a-32 16SrRNA	99%
C14	canola	SV	*Pseudomonas* sp. SBV1 16SrRNA	99%
C15	canola	SV	*Pseudomonas* sp. AC-167 16SrRNA	99%
C16	canola	SV	*Pseudomonas* sp. SBV1 16SrRNA	99%
C17	canola	SV	Uncultured bacterium clone Upland_120_2953 16SrRNA	99%
C18	canola	SV	*Pseudomonas* sp. F330-7 16SrRNA	99%
C19	canola	SV	Uncultured bacterium clone Upland_120_2953 16SrRNA	99%
C20	canola	SN	*Pantoea vagans* strain Eb-2 16SrRNA	81%
C21	canola	SN	Uncultured bacterium clone Upland_120_2953 16SrRNA	97%
C22	canola	SN	Uncultured bacterium clone Upland_500_9740 16SrRNA	92%
C23	canola	SN	*Pseudomonas arsenicoxydans* partial 16SrRNA, isolate SKPB2	98%
C24	canola	SN	Uncultured bacterium clone 27 16SrRNA	98%
C25	canola	MF	*Pseudomonas* sp. BG2dil partial 16SrRNA, isolate M2	99%
C26	canola	MF	Uncultured bacterium clone Upland_120_2953 16SrRNA	93%
C27	canola	MF	*Stenotrophomonas* sp. Artichoke A1 16SrRNA	97%
C28	canola	MF	Uncultured bacterium clone Upland_120_2953 16SrRNA	99%
W1	wheat	CB	*Pseudomonas* sp. Y4_286_1 16SrRNA	94%
W2	wheat	CB	*Flavobacterium* sp. OR306 16SrRNA	99%
W3	wheat	CB	*Klebsiella pneumoniae* strain CWS1 16SrRNA	92%

ID	Crop	Location	Closest match	Similarity (%)
W4	wheat	CB	Uncultured bacterium clone ncd2052d12c2 16S rRNA	95%
W5	wheat	CB	*Pseudomonas koreensis* partial 16S rRNA, isolate 0511TES17Q4	99%
W6	wheat	CB	*Pantoea agglomerans* strain EQH21 16S rRNA	99%
W7	wheat	CB	Uncultured bacterium isolate DGGE gel band 2a 16S rRNA	88%
W8	wheat	CB	*Streptomyces* sp. AK02-1a 16S rRNA	91%
W9	wheat	CB	*Streptomyces* sp. 2-G 16S rRNA	99%
W10	wheat	CB	*Arthrobacter* sp. B2031 16S rRNA	95%
W11	wheat	CB	*Streptomyces* sp. MSSRFAF8 16S rRNA	98%
W12	wheat	CB	*Arthrobacter* sp. 3B5-2009 16S rRNA	90%
W13	wheat	SN	*Arthrobacter* sp. 3B5-2009 16S rRNA	92%
W14	wheat	SN	*Stenotrophomonas rhizophila* strain HED03 16S rRNA	90%
W15	wheat	SN	*Arthrobacter* sp. B2031 16S rRNA	92%
W16	wheat	SN	*Arthrobacter globiformis* partial 16S rRNA, isolate 0312MAR1A6	91%
W17	wheat	MF	Uncultured bacterium for 16S rRNA, clone: OYMC-Endo-CLN27	98%
W18	wheat	MF	Uncultured bacterium for 16S rRNA, clone: OYMC-Endo-CLN27	99%
W19	wheat	MF	*Flavobacterium* sp. R-38295 partial 16S rRNA, strain R-38295	96%
W20	wheat	MF	Uncultured bacterium for 16S rRNA, clone: OYMC-Endo-CLN27	99%
W21	wheat	MF	Uncultured bacterium for 16S rRNA, clone: OYMC-Endo-CLN27	99%
L1	lentil	CB	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L2	lentil	CB	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L3	lentil	CB	*Rhizobium* sp. BLR59 16S rRNA	99%
L4	lentil	CB	*Rhizobium leguminosarum* strain PB173 16S rRNA	99%
L5	lentil	CB	*Rhizobium* sp. FYRM59 16S rRNA	99%
L6	lentil	CB	*Rhizobium* sp. NisB-1 16S rRNA	99%
L7	lentil	CB	*Rhizobium* sp. NisB-1 16S rRNA	99%
L8	lentil	CB	*Rhizobium* sp. FYRM58 16S rRNA	99%
L9	lentil	CB	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L10	lentil	CB	Uncultured clone ELU0032-T156-S-NIPCRAMgANb_000115 16S rRNA	99%
L11	lentil	CB	Uncultured bacterium clone FL11e10_16137 16S rRNA	99%
L12	lentil	CB	Uncultured bacterium clone FL11e10_16137 16S rRNA	98%
L13	lentil	CB	Uncultured clone ELU0032-T156-S-NIPCRAMgANb_000115 16S rRNA	99%
L14	lentil	CB	*Rhizobium* sp. NisB-1 16S rRNA	99%
L15	lentil	CB	*Rhizobium* sp. FYRM58 16S rRNA	99%
L16	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%
L17	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%

ID	Crop	Location	Closest match	Similarity (%)
L18	lentil	SV	Uncultured bacterium clone Upland_120_2953 16S rRNA	93%
L19	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%
L20	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%
L21	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%
L22	lentil	SV	*Rhizobium* sp. NCHA22 16S rRNA	99%
L23	lentil	SV	*Rhizobium* sp. BLR59 16S rRNA	99%
L24	lentil	SV	*Rhizobium* sp. CTG-423 16S rRNA	99%
L25	lentil	SV	*Rhizobium* sp. CTG-423 16S rRNA	99%
L26	lentil	SV	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L27	lentil	SV	Uncultured bacterium clone FL11e10_16137 16S rRNA	96%
L28	lentil	SV	*Rhizobium* sp. NisB-1 16S rRNA	99%
L29	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L30	lentil	SN	*Rhizobium* sp. NCHA22 16S rRNA	99%
L31	lentil	SN	*Rhizobium* sp. NCHA22 16S rRNA	99%
L32	lentil	SN	*Rhizobium* sp. NCHA22 16S rRNA	99%
L33	lentil	SN	*Rhizobium* sp. NCHA22 16S rRNA	99%
L34	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L35	lentil	SN	*Rhizobium* sp. CTG-423 16S rRNA	99%
L36	lentil	SN	*Rhizobium* sp. FYRM58 16S rRNA	99%
L37	lentil	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
L38	lentil	SN	Uncultured bacterium clone FL11e10_16137 16S rRNA	99%
L39	lentil	SN	*Rhizobium* sp. FYRM58 16S rRNA	99%
L40	lentil	SN	*Rhizobium* sp. FYRM59 16S rRNA	99%
P1	pea	CB	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	100%
P2	pea	CB	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	98%
P3	pea	CB	*Rhizobium* sp. FYRM58 16S rRNA	99%
P4	pea	CB	*Rhizobium etli* EBRI 21 16S rRNA	99%
P5	pea	CB	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	97%
P6	pea	SV	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	98%
P7	pea	SV	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	100%
P8	pea	SV	*Rhizobium* sp. NisB-1 16S rRNA	99%
P9	pea	SV	Uncultured Rhizobium sp. clone GASP-MB2W3_D11 16S rRNA	99%
P10	pea	SV	Uncultured bacterium for 16S rRNA , clone: OYMC-Endo-CLN03	98%
P11	pea	SN	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	98%
P12	pea	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	100%

ID	Crop	Location	Closest match	Similarity (%)
P13	pea	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	100%
P14	pea	SN	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	98%
P15	pea	MF	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
P16	pea	MF	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	99%
P17	pea	MF	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	98%
P18	pea	MF	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	100%
P19	pea	MF	Uncultured bacterium clone A04778Aco 16S rRNA	99%
P20	pea	MF	*Rhizobium leguminosarum* strain 6.1.1 16S rRNA	99%
P21	pea	MF	*Rhizobium* sp. NCHA22 16S rRNA	99%
P22	pea	MF	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	99%
P23	pea	MF	*Rhizobium leguminosarum* bv. *viciae* strain MMUST-003 16S rRNA	98%

APPENDIX C: Canola and wheat grown at agricultural potted Brown and Black soils from Central Butte and Melfort, Saskatchewan at stem elongation, flowering and ripening

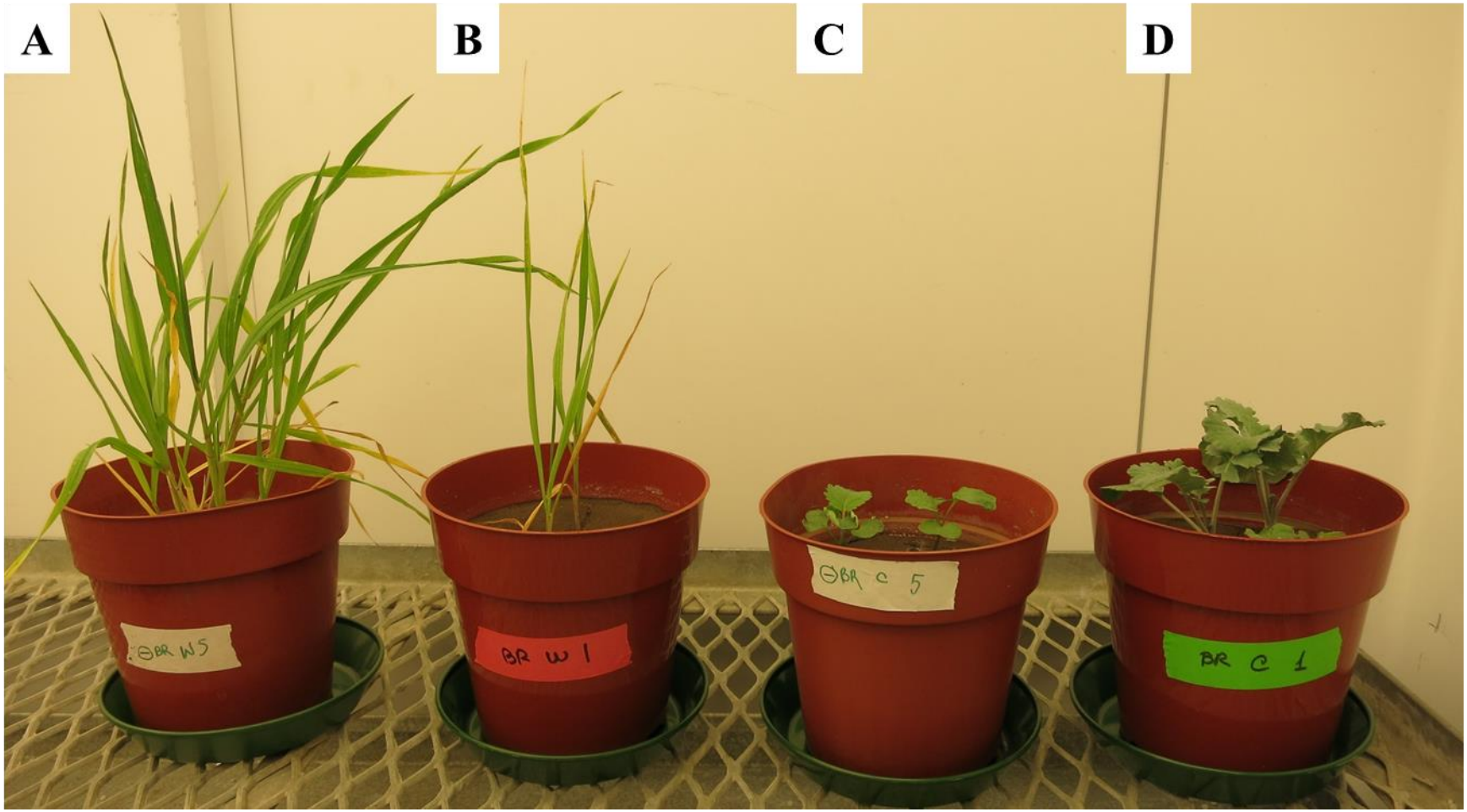

Fig. C.1. Wheat (A, B) and canola (C, D) grown in an agricultural potted Brown soil from Central Butte, Saskatchewan. Plants were harvested at stem elongation.

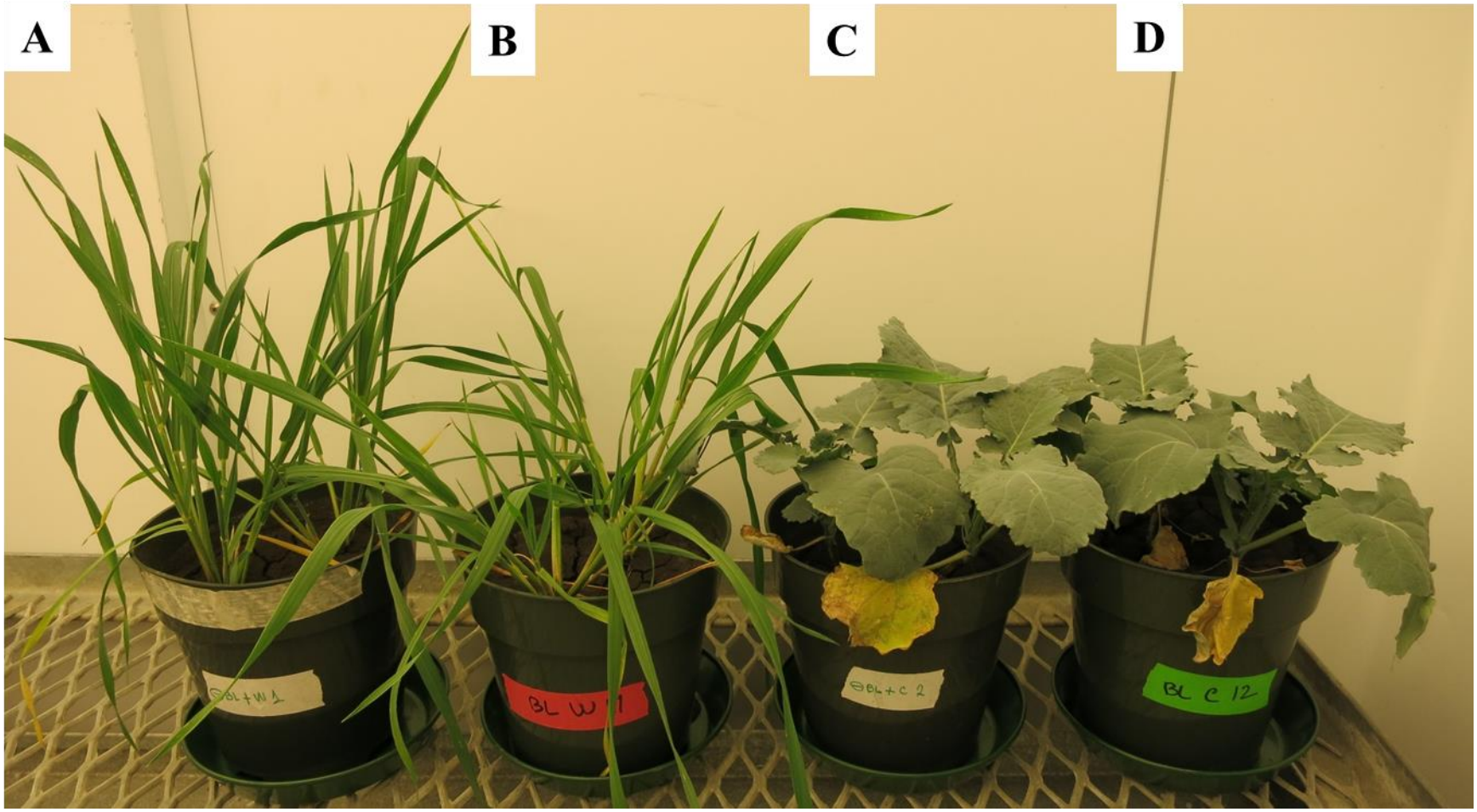

Fig. C.2. Wheat (A, B) and canola (C, D) grown in an agricultural potted Black soil from Melfort, Saskatchewan. Plants were harvested at stem elongation.

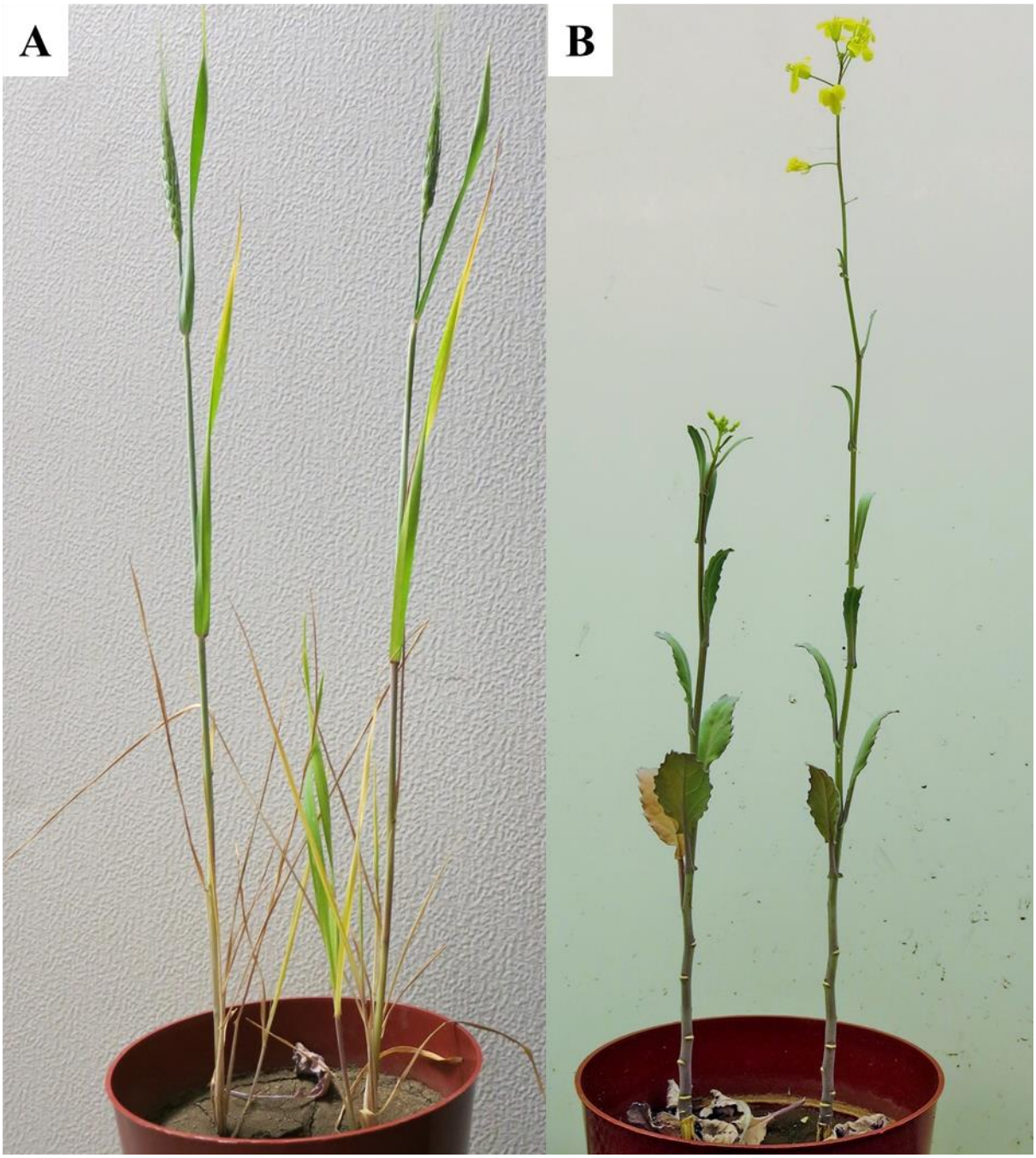

Fig. C.3. Wheat (A) and canola (B) grown in an agricultural potted Brown soil from Central Butte, Saskatchewan. Plants were harvested at flowering.

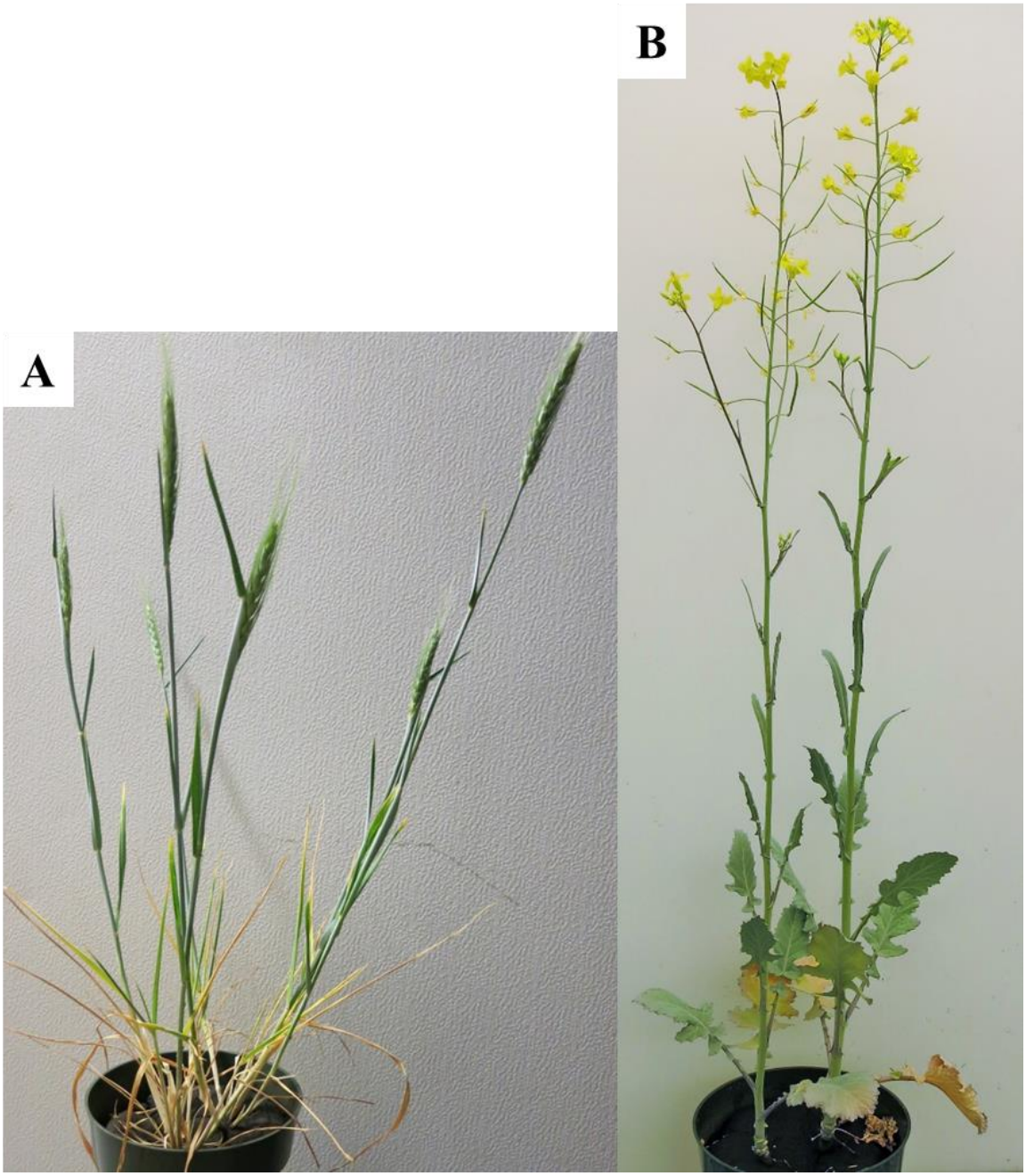

Fig. C.4. Wheat (A) and canola (B) grown in an agricultural potted Black soil from Melfort, Saskatchewan. Plants were harvested at flowering.

Fig. C.5. Wheat (A) and canola (B) grown in an agricultural potted Brown soil from Central Butte, Saskatchewan. Plants were harvested at ripening.

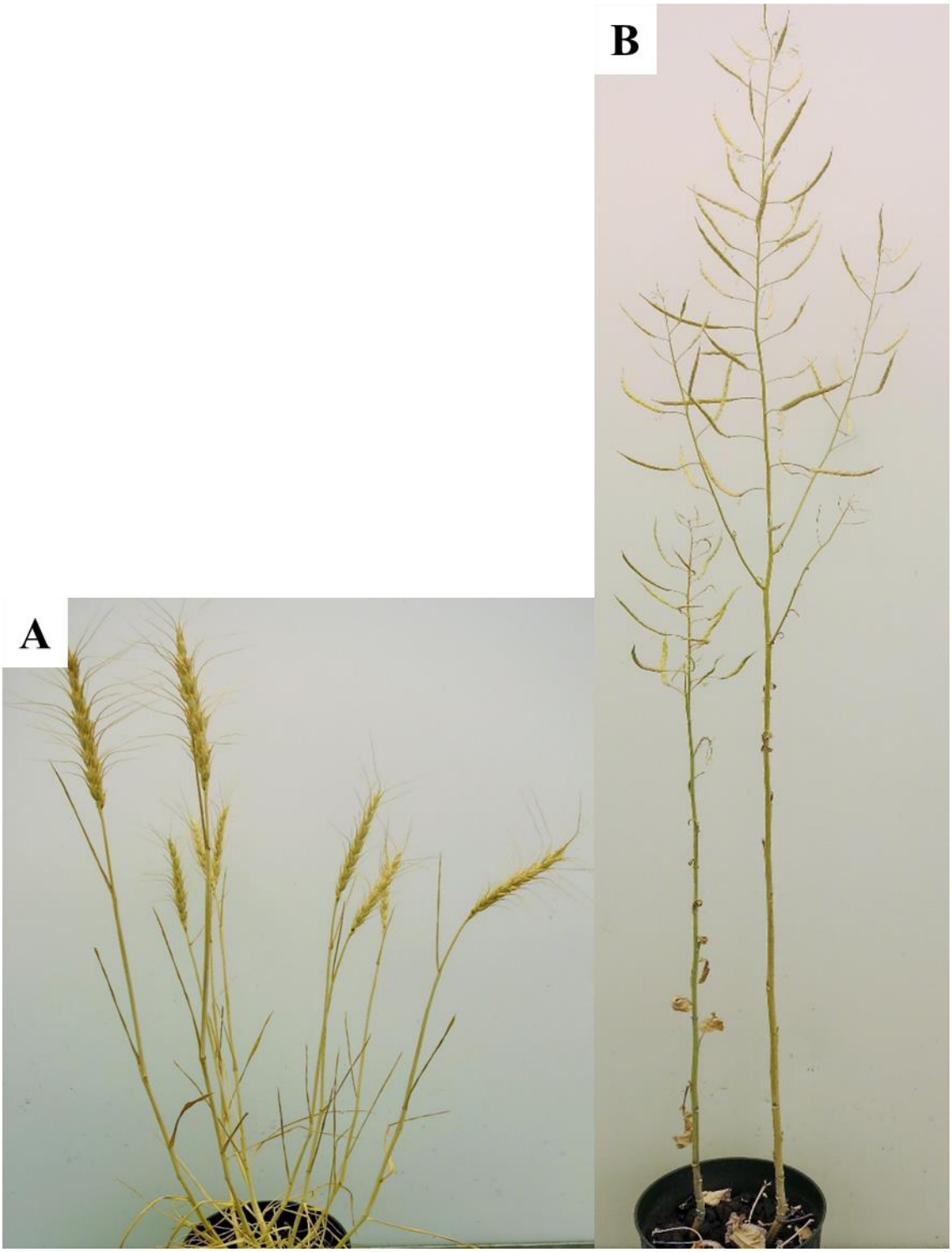

Fig. C.6. Wheat (A) and canola (B) grown in an agricultural potted Black soil from Melfort, Saskatchewan. Plants were harvested at ripening.

APPENDIX D: Seed germination rates of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from crops grown in Saskatchewan

Table D.1. Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from canola roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
control		32.5	5.0		45.0	12.9		80.0	11.5		30.0	11.5		82.5	9.6		62.5	9.6		95.0	5.8		62.5	12.6	
CS1_4	*Acinetobacter calcoaceticus*	75.0	20.8	↑	35.0	12.9		37.5	17.1	↓	35.0	31.1		92.5	9.6		50.0	8.2		72.5	17.1		75.0	17.3	
CS1_5	*Acinetobacter calcoaceticus*	40.0	18.3		50.0	8.2		10.0	14.1	↓	25.0	5.8		80.0	18.3		70.0	8.2		47.5	15.0	↓	70.0	8.2	
CS1_6	*Acinetobacter calcoaceticus*	32.5	5.0		45.0	19.1		17.5	9.6	↓	10.0	14.1		77.5	5.0		50.0	14.1		80.0	18.3		85.0	19.1	↑
CS1_16	*Acinetobacter calcoaceticus*	32.5	20.6		40.0	18.3		57.5	15.0		20.0	8.2		87.5	12.6		62.5	25.0		87.5	9.6		67.5	29.9	
CS1_18	*Acinetobacter calcoaceticus*	35.0	10.0		65.0	12.9	↑	17.5	9.6	↓	20.0	21.6		90.0	8.2		75.0	12.9		70.0	8.2		55.0	19.1	
CCB1_7	*Arthrobacter phenanthrenivorans*	35.0	10.0		35.0	23.8		40.0	16.3	↓	20.0	8.2		92.5	9.6		60.0	29.4		82.5	22.2		82.5	9.6	
CS1_10	*Bacillus abyssalis*	32.5	15.0		50.0	8.2		50.0	16.3		27.5	20.6		82.5	15.0		60.0	14.1		90.0	0.0		85.0	23.8	↑
CS1_7	*Bacillus aryabhattai*	30.0	8.2		22.5	15.0	↓	65.0	20.8		37.5	23.6		82.5	23.6		40.0	24.5		95.0	10.0		77.5	12.6	
CS1_8	*Bacillus aryabhattai*	7.5	5.0	↓	32.5	5.0		72.5	9.6		47.5	26.3		92.5	9.6		50.0	11.5		97.5	5.0		85.0	19.1	↑
CS1_11	*Bacillus aryabhattai*	62.5	20.6	↑	67.5	12.6	↑	80.0	8.2		20.0	11.5		92.5	9.6		90.0	11.5		95.0	10.0	↑	80.0	14.1	
CS1_9	*Bacillus halosaccharovorans*	7.5	9.6	↓	30.0	18.3		52.5	9.6		27.5	5.0		70.0	18.3		55.0	12.9		87.5	12.6		70.0	21.6	
CS1_12	*Bacillus halosaccharovorans*	72.5	12.6	↑	40.0	11.5		70.0	14.1		32.5	22.2		92.5	5.0		57.5	18.9		90.0	14.1		77.5	20.6	
CCB1_5	*Brucella ceti*	77.5	9.6	↑	40.0	18.3		27.5	17.1	↓	32.5	20.6		97.5	5.0	↑	57.5	12.6		90.0	14.1		87.5	9.6	↑
CCB1_25	*Brucella ceti*	47.5	12.6		32.5	9.6		32.5	15.0	↓	7.5	9.6		80.0	8.2		40.0	0.0		82.5	17.1		55.0	17.3	
CM3_17	*Chryseobacterium humi*	55.0	26.5	↑	60.0	8.2		47.5	17.1	↓	20.0	0.0		92.5	5.0		67.5	15.0		92.5	9.6		72.5	5.0	
CM3_12	*Neisseria flava*	37.5	20.6		27.5	5.0		65.0	12.9		27.5	15.0		82.5	15.0		47.5	22.2		75.0	17.3		67.5	32.0	
CS1_1	*Pantoea vagans*	75.0	12.9	↑	60.0	18.3		70.0	14.1		25.0	5.8		90.0	14.1		75.0	12.9		97.5	5.0		55.0	20.8	
CS1_2	*Pantoea vagans*	65.0	12.9	↑	55.0	10.0		82.5	5.0		22.5	22.2		97.5	5.0	↑	72.5	20.6		100.0	0.0		67.5	22.2	
CS1_3	*Pantoea vagans*	47.5	9.6		52.5	12.6		65.0	5.8		30.0	16.3		77.5	9.6		75.0	5.8		95.0	5.8		65.0	12.9	
CS1_13	*Pseudomonas mediterranea*	32.5	5.0		20.0	8.2	↓	67.5	17.1		25.0	5.8		80.0	16.3		37.5	12.6		87.5	9.6		55.0	5.8	
CS1_14	*Pseudomonas mediterranea*	40.0	14.1		15.0	5.8	↓	62.5	18.9		30.0	14.1		82.5	12.6		32.5	9.6		95.0	5.8		52.5	23.6	
CM3_9	*Pseudomonas monteilii*	30.0	8.2		15.0	5.8	↓	82.5	9.6		35.0	17.3		77.5	12.6		52.5	12.6		97.5	5.0		80.0	14.1	
CS1_15	*Pseudomonas poae*	25.0	12.9		22.5	5.0	↓	50.0	24.5		10.0	0.0	↓	82.5	5.0		47.5	12.6		92.5	15.0		37.5	12.6	
CK4_8	*Pseudomonas tolaasii*	17.5	9.6		25.0	5.8		90.0	14.1	↑	60.0	21.6	↑	80.0	16.3		65.0	12.9		92.5	15.0		90.0	14.1	↑

Table D.1. (cont.). Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from canola roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
CM3_8	*Pseudoxanthomonas koreensis*	12.5	5.0		40.0	14.1		45.0	12.9	↓	15.0	12.9		92.5	9.6		45.0	17.3		87.5	12.6		52.5	18.9	
CM3_13	*Pseudoxanthomonas taiwanensis*	40.0	23.1		37.5	17.1		67.5	9.6		27.5	5.0		95.0	10.0		62.5	26.3		92.5	5.0		95.0	5.8	↑
CCB1_8	*Serratia liquefaciens*	45.0	12.9		27.5	12.6		30.0	18.3	↓	45.0	5.8		92.5	9.6		42.5	22.2		85.0	12.9		80.0	18.3	
CCB1_9	*Stenotrophomonas maltophilia*	35.0	5.8		47.5	28.7		60.0	14.1		25.0	17.3		92.5	9.6		60.0	28.3		92.5	5.0		65.0	19.1	
CCB1_10	*Stenotrophomonas maltophilia*	25.0	5.8		50.0	14.1		45.0	19.1	↓	40.0	8.2		85.0	17.3		75.0	17.3		77.5	9.6		77.5	17.1	
CCB1_12	*Stenotrophomonas maltophilia*	22.5	5.8		50.0	14.1		65.0	19.1		40.0	8.2		85.0	17.3		60.0	17.3		97.5	9.6		65.0	17.1	
CCB1_22	*Stenotrophomonas maltophilia*	75.0	5.8	↑	52.5	17.1		77.5	9.6		12.5	9.6		95.0	5.8		55.0	20.8		100.0	0.0		57.5	15.0	
CCB1_23	*Stenotrophomonas maltophilia*	32.5	17.1		52.5	18.9		67.5	5.0		32.5	15.0		77.5	15.0		52.5	18.9		90.0	8.2		67.5	9.6	
CCB1_24	*Stenotrophomonas maltophilia*	20.0	18.3		57.5	12.6		50.0	24.5		32.5	26.3		90.0	8.2		75.0	5.8		87.5	12.6		75.0	17.3	
CM3_1	*Stenotrophomonas maltophilia*	27.5	9.6		70.0	14.1	↑	52.5	15.0		30.0	14.1		75.0	12.9		77.5	12.6		97.5	5.0		85.0	10.0	
CM3_15	*Stenotrophomonas maltophilia*	75.0	12.9	↑	50.0	8.2		60.0	11.5		17.5	23.6		97.5	5.0	↑	62.5	15.0		95.0	5.8		47.5	29.9	
CM3_16	*Stenotrophomonas maltophilia*	30.0	8.2		42.5	15.0		42.5	20.6	↓	20.0	18.3		87.5	18.9		55.0	12.9		92.5	9.6		60.0	25.8	
CM3_18	*Stenotrophomonas maltophilia*	22.5	12.6		35.0	5.8		62.5	12.6		22.5	12.6		87.5	12.6		52.5	15.0		95.0	10.0		62.5	12.6	
CM3_3	*Stenotrophomonas maltophilia*	50.0	0.0		32.5	17.1		52.5	9.6		32.5	22.2		85.0	12.9		47.5	25.0		90.0	8.2		65.0	12.9	
CM3_4	*Stenotrophomonas maltophilia*	17.5	12.6		30.0	16.3		45.0	12.9	↓	10.0	8.2	↓	87.5	18.9		65.0	23.8		95.0	10.0		37.5	12.6	
CM3_7	*Stenotrophomonas maltophilia*	40.0	8.2		45.0	5.8		57.5	22.2		32.5	12.6		90.0	8.2		50.0	0.0		82.5	12.6		70.0	14.1	
CM3_6	*Stenotrophomonas* sp.	20.0	14.1		45.0	10.0		60.0	14.1		10.0	20.0		87.5	5.0		47.5	9.6		92.5	9.6		60.0	8.2	
CS1_17	*Xanthomonas fuscans*	15.0	5.8		32.5	18.9		57.5	15.0		25.0	19.1		85.0	12.9		57.5	23.6		95.0	5.8		77.5	5.0	

Table D.2. Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from wheat roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
control		32.5	5.0		45.0	12.9		80.0	11.5		30.0	11.5		82.5	9.6		62.5	9.6		95.0	5.8		62.5	12.6	
WCB1_23	*Agrococcus carbonis*	37.5	5.0		27.5	17.1		65.0	17.3		37.5	15.0		100.0	0.0	↑	65.0	10.0		95.0	5.8		95.0	5.8	↑
WCB1_6	*Agrococcus citreus*	30.0	8.2		57.5	12.6		72.5	15.0		32.5	15.0		95.0	5.8		67.5	18.9		97.5	5.0		85.0	17.3	↑
WCB1_13	*Arthrobacter subterraneus*	37.5	17.1		40.0	14.1		72.5	17.1		32.5	5.0		90.0	8.2		52.5	15.0		95.0	5.8		57.5	9.6	
WCB1_1	*Brevibacillus agri*	35.0	20.8		50.0	8.2		80.0	14.1		17.5	12.6		95.0	10.0	↑	52.5	9.6		97.5	5.0		67.5	18.9	
WCB1_2	*Brevibacillus agri*	15.0	12.9		47.5	22.2		85.0	12.9		2.5	5.0	↓	92.5	15.0		75.0	26.5		87.5	15.0		40.0	21.6	
WCB1_3	*Brevibacillus agri*	42.5	5.0		35.0	12.9		85.0	5.8		2.5	5.0	↓	92.5	9.6		57.5	15.0		97.5	5.0		57.5	20.6	
WCB1_5	*Brevibacillus agri*	32.5	17.1		25.0	10.0		95.0	5.8	↑	5.0	5.8	↓	95.0	5.8		47.5	5.0		95.0	5.8		60.0	8.2	
WCB2_10	*Brevibacillus agri*	30.0	8.2		50.0	20.0		87.5	5.0		0.0	0.0	↓	90.0	0.0		70.0	18.3		95.0	5.8		47.5	20.6	
WCB2_12	*Brevibacillus agri*	35.0	19.1		32.5	18.9		80.0	14.1		5.0	5.8	↓	90.0	8.2		55.0	17.3		95.0	10.0		55.0	30.0	
WMF1_2	*Erwinia tasmaniensis*	40.0	18.3		47.5	12.6		92.5	9.6		20.0	8.2		82.5	5.0		67.5	17.1		100.0	0.0		70.0	8.2	
WMF1_3	*Erwinia tasmaniensis*	20.0	14.1		27.5	17.1		82.5	12.6		0.0	0.0	↓	85.0	5.8		47.5	17.1		100.0	0.0		70.0	23.1	
WMF1_4	*Erwinia tasmaniensis*	30.0	8.2		40.0	11.5		75.0	10.0		17.5	17.1		87.5	15.0		57.5	9.6		100.0	0.0		67.5	5.0	
WMF1_8	*Erwinia tasmaniensis*	17.5	5.0		12.5	5.0		77.5	22.2		0.0	0.0	↓	82.5	15.0		27.5	9.6	↓	100.0	0.0		35.0	17.3	
WMF2_8	*Galbitalea soli*	30.0	14.1		45.0	17.3		57.5	15.0		2.5	5.0	↓	85.0	10.0		52.5	12.6		95.0	5.8		52.5	12.6	
WMF2_9	*Galbitalea soli*	47.5	12.6		37.5	9.6		87.5	9.6		10.0	0.0		100.0	0.0	↑	50.0	11.5		97.5	5.0		67.5	17.1	
WCB1_7	*Kinneretia asaccharophila*	10.0	8.2		15.0	12.9		92.5	5.0		2.5	5.0	↓	77.5	9.6		40.0	16.3		100.0	0.0		27.5	15.0	↓
WMF1_5	*Leifsonia xyli*	77.5	22.2	↑	40.0	20.0		77.5	20.6		40.0	16.3		95.0	5.8		60.0	8.2		97.5	5.0		87.5	5.0	↑
WMF1_6	*Leifsonia xyli*	70.0	18.3	↑	37.5	12.6		67.5	9.6		47.5	9.6		92.5	5.0		55.0	5.8		92.5	5.0		82.5	15.0	
WMF1_7	*Leifsonia xyli*	77.5	5.0	↑	25.0	12.9		60.0	18.3		22.5	15.0		97.5	5.0	↑	55.0	31.1		100.0	0.0		65.0	28.9	
WCB2_13	*Luteimonas huabeiensis*	22.5	9.6		25.0	12.9		77.5	12.6		2.5	5.0	↓	87.5	9.6		42.5	9.6		100.0	0.0		52.5	22.2	
WMF2_7	*Mesorhizobium australicum*	40.0	18.3		25.0	10.0		85.0	10.0		0.0	0.0	↓	87.5	9.6		52.5	22.2		95.0	10.0		12.5	12.6	↓

Table D.2. (cont.). Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from wheat roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
WMF2_4	*Microbacterium saccharophilum*	7.5	5.0	↓	55.0	19.1		50.0	14.1		40.0	14.1		87.5	12.6		75.0	5.8		95.0	10.0		82.5	15.0	
WMF2_5	*Microbacterium saccharophilum*	15.0	12.9		37.5	9.6		67.5	22.2		20.0	14.1		90.0	8.2		52.5	9.6		100.0	0.0		80.0	20.0	
WMF2_6	*Microbacterium saccharophilum*	22.5	9.6		42.5	5.0		67.5	17.1		42.5	12.6		95.0	5.8		57.5	17.1		100.0	0.0		97.5	5.0	↑
WK1_2	*Microbacterium* sp.	47.5	18.9		52.5	17.1		60.0	8.2		0.0	0.0	↓	92.5	9.6		65.0	12.9		100.0	0.0		50.0	8.2	
WK1_12	*Microbacterium sp.*	40.0	31.6		42.5	17.1		55.0	10.0		32.5	17.1		87.5	9.6		50.0	25.8		87.5	15.0	↑	65.0	17.3	
WK1_3	*Microbacterium sp.*	47.5	15.0		35.0	12.9		75.0	20.8		2.5	5.0	↓	82.5	15.0		45.0	12.9		97.5	5.0		40.0	18.3	
WK1_4	*Microbacterium sp.*	32.5	5.0		32.5	15.0		90.0	11.5		32.5	9.6		87.5	12.6		47.5	5.0		97.5	5.0		80.0	21.6	
WCB1_14	*Mycetocola manganoxydans*	70.0	24.5	↑	42.5	18.9		80.0	11.5		45.0	5.8		92.5	9.6		57.5	9.6		95.0	5.8		90.0	8.2	↑
WCB2_3	*Mycetocola zhadangensis*	75.0	12.9	↑	40.0	14.1		75.0	5.8		22.5	5.0		90.0	8.2		65.0	10.0		92.5	5.0		87.5	12.6	↑
WMF2_3	*Mycobacterium smegmatis*	62.5	22.2	↑	47.5	9.6		87.5	9.6		20.0	14.1		87.5	9.6		57.5	17.1		100.0	0.0		72.5	9.6	
WMF2_10	*Mycobacterium smegmatis*	52.5	20.6		60.0	8.2		82.5	5.0		15.0	5.8		90.0	8.2		75.0	12.9		90.0	8.2		52.5	12.6	
WMF2_11	*Mycobacterium smegmatis*	45.0	17.3		47.5	15.0		65.0	12.9		32.5	20.6		92.5	9.6		60.0	8.2		97.5	5.0		77.5	17.1	
WMF2_12	*Mycobacterium smegmatis*	65.0	20.8	↑	70.0	11.5	↑	85.0	5.8		22.5	12.6		92.5	9.6		75.0	10.0		97.5	5.0		65.0	12.9	
WCB2_2	*Paenibacillus taohuashanense*	25.0	10.0		40.0	8.2		77.5	12.6		7.5	9.6		100.0	0.0	↑	55.0	12.9		100.0	0.0		57.5	40.3	
WCB2_4	*Paenibacillus taohuashanense*	22.5	12.6		25.0	12.9		92.5	9.6		37.5	23.6		90.0	8.2		45.0	10.0		100.0	0.0		80.0	16.3	
WCB2_18	*Pseudomonas fluorescens*	2.5	5.0	↓	20.0	14.1	↓	25.0	31.1	↓	7.5	5.0	↓	87.5	12.6		85.0	19.1		100.0	0.0		32.5	20.6	
WCB2_5	*Pseudomonas fluorescens*	0.0	0.0	↓	2.5	5.0	↓	10.0	8.2	↓	2.5	5.0	↓	90.0	8.2		50.0	18.3		95.0	5.8		12.5	5.0	↓
WCB2_7	*Pseudomonas fluorescens*	5.0	10.0	↓	7.5	9.6	↓	15.0	5.8	↓	12.5	5.0	↓	82.5	9.6		57.5	12.6		97.5	5.0		45.0	12.9	
WCB2_8	*Pseudomonas fluorescens*	2.5	5.0	↓	2.5	5.0	↓	12.5	9.6	↓	7.5	5.0	↓	92.5	5.0		47.5	9.6		100.0	0.0		32.5	17.1	
WCB2_9	*Pseudomonas fluorescens*	2.5	5.0	↓	0.0	0.0	↓	37.5	5.0	↓	12.5	9.6		92.5	9.6		45.0	5.8		95.0	5.8		42.5	20.6	

Table D.2. (cont.). Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from wheat roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
WCB1_16	*Rhizobium skierniewicense*	35.0	5.8		30.0	8.2		52.5	12.6		5.0	5.8	↓	90.0	8.2		45.0	25.2		97.5	5.0		37.5	17.1	
WCB2_11	*Rhizobium skierniewicense*	32.5	18.9		37.5	15.0		62.5	15.0		2.5	5.0	↓	90.0	11.5		55.0	19.1		90.0	11.5		15.0	5.8	↓
WMF1_1	*Rhizobium skierniewicense*	30.0	14.1		37.5	22.2		82.5	9.6		5.0	10.0	↓	90.0	8.2		60.0	14.1		100.0	0.0		35.0	12.9	
WCB1_9	*Rhizobium sp.*	50.0	16.3		32.5	9.6		55.0	10.0		0.0	0.0	↓	95.0	5.8		60.0	16.3		90.0	11.5		15.0	5.8	↓
WCB1_10	*Rhodococcus cerastrii*	47.5	12.6		45.0	5.8		57.5	17.1		7.5	5.0	↓	100.0	0.0	↑	52.5	9.6		87.5	5.0		75.0	17.3	
WCB1_11	*Rhodococcus cercidiphylli*	27.5	9.6		17.5	15.0		70.0	18.3		10.0	8.2		90.0	11.5		37.5	18.9		95.0	5.8		67.5	5.0	
WCB1_12	*Rhodococcus sp.*	7.5	9.6	↓	7.5	5.0	↓	30.0	14.1	↓	5.0	5.8	↓	85.0	5.8		40.0	14.1		90.0	11.5		50.0	21.6	
WK1_10	*Stenotrophomonas pavanii*	42.5	12.6		37.5	9.6		82.5	9.6		37.5	28.7		92.5	9.6		57.5	9.6		100.0	0.0		87.5	9.6	↓
WCB2_14	*Stenotrophomonas rhizophila*	22.5	9.6		22.5	26.3		77.5	5.0		15.0	5.8		97.5	5.0	↑	35.0	26.5		97.5	5.0		42.5	5.0	
WCB2_15	*Stenotrophomonas rhizophila*	30.0	18.3		32.5	12.6		95.0	10.0	↑	15.0	5.8		82.5	12.6		40.0	14.1		100.0	0.0		32.5	12.6	
WCB1_24	*Streptomyces griseus*	15.0	5.8		37.5	12.6		72.5	18.9		2.5	5.0	↓	90.0	11.5		62.5	22.2		100.0	0.0		42.5	15.0	
WCB1_8	*Veillonellaceae*	65.0	5.8	↑	47.5	9.6		75.0	10.0		7.5	5.0		90.0	14.1		62.5	9.6		92.5	9.6		67.5	22.2	
WCB1_17	*Williamsia marianensis*	52.5	18.9		50.0	18.3		77.5	15.0		7.5	9.6		90.0	8.2		62.5	15.0		92.5	9.6		65.0	10.0	
WCB1_20	*Williamsia marianensis*	55.0	17.3	↑	60.0	14.1		77.5	12.6		25.0	10.0		90.0	0.0		80.0	14.1		97.5	5.0		75.0	12.9	
WCB1_22	*Williamsia marianensis*	35.0	12.9		42.5	18.9		62.5	18.9		2.5	5.0	↓	90.0	11.5		45.0	17.3		100.0	0.0		20.0	14.1	↓
WK1_11	*Xanthomonas campestris*	17.5	12.6		27.5	9.6		85.0	9.6		7.5	28.7		92.5	9.6		37.5	9.6		100.0	0.0		35.0	9.6	
WK1_4	*Xanthomonas fuscans*	32.5	12.6		35.0	19.1		80.0	16.3		10.0	14.1	↓	95.0	5.8		55.0	12.9		97.5	5.0		30.0	14.1	
WK1_5	*Xanthomonas fuscans*	35.0	12.9		17.5	17.1		82.5	9.6		12.5	18.9		97.5	5.0	↑	30.0	21.6		100.0	0.0		25.0	17.3	
WK1_6	*Xanthomonas fuscans*	65.0	12.9	↑	27.5	22.2		92.5	9.6		17.5	15.0		95.0	10.0	↑	50.0	24.5		100.0	0.0		25.0	12.9	↓
WK1_7	*Xanthomonas fuscans*	42.5	20.6		7.5	5.0	↓	77.5	22.2		20.0	21.6		90.0	14.1		40.0	11.5		100.0	0.0		32.5	20.6	
WK1_8	*Xanthomonas fuscans*	40.0	21.6		20.0	8.2		75.0	10.0		37.5	33.0		82.5	9.6		40.0	14.1		90.0	0.0		67.5	40.3	
WK1_9	*Xanthomonas fuscans*	37.5	15.0		25.0	17.3		82.5	5.0		10.0	8.2		85.0	23.8		40.0	18.3		87.5	5.0		52.5	5.0	

Table D.3. Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from pea roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, P≤0.05.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D		x̄	S.D	
control		32.5	5.0		45.0	12.9		80.0	11.5		30.0	11.5		82.5	9.6		62.5	9.6		95.0	5.8		62.5	12.6	
P SV1-11	*Bacillus halosaccharovorans*	82.5	9.6	↑	45.0	19.1		60.0	21.6		17.5	15.0		95.0	5.8	↑	50.0	18.3		82.5	17.1		60.0	8.2	
P SV1-14	*Bacillus halosaccharovorans*	5.0	5.8		37.5	25.0		67.5	15.0		22.5	22.2		77.5	15.0		57.5	15.0		87.5	9.6		75.0	26.5	
P SV1-16	*Bacillus halosaccharovorans*	35.0	28.9		27.5	12.6		30.0	14.1	↓	12.5	12.6		90.0	8.2		47.5	9.6		65.0	30.0		52.5	15.0	↓
P K4-6	*Bacillus halosaccharovorans*	32.5	9.6		37.5	5.0		35.0	5.8	↓	10.0	8.2		80.0	8.2		62.5	5.0		95.0	10.0		25.0	12.9	
P K1-10	*Bacillus halosaccharovorans*	70.0	14.1	↑	30.0	8.2		75.0	5.8		12.5	9.6		90.0	8.2		52.5	20.6		85.0	12.9		65.0	17.3	
P K1-12	*Bacillus halosaccharovorans*	40.0	8.2		30.0	14.1		57.5	12.6	↓	35.0	17.3		92.5	5.0		45.0	12.9		85.0	12.9		77.5	17.1	
P SV1-15	*Paenibacillus pabuli*	65.0	17.3	↑	52.5	12.6		22.5	9.6	↓	5.0	10.0	↓	77.5	9.6		55.0	12.9		22.5	9.6	↓	25.0	17.3	↓
P SV1-7	*Pantoea agglomerans*	70.0	24.5	↑	72.5	25.0	↑	50.0	14.1	↓	12.5	9.6		97.5	5.0	↑	77.5	26.3	↑	85.0	5.8		57.5	20.6	
P MF3-1	*Pantoea vagans*	62.5	20.6		27.5	9.6		27.5	17.1	↓	22.5	9.6		100.0	0.0	↑	40.0	8.2		85.0	10.0		65.0	12.9	
P MF3-3	*Pantoea vagans*	85.0	12.9	↑	37.5	22.2		50.0	8.2	↓	17.5	9.6		97.5	5.0	↑	52.5	12.6		97.5	5.0		60.0	8.2	
P CB1-13	*Pseudomonas mucidolens*	22.5	25.0		37.5	27.5		27.5	9.6	↓	20.0	24.5		77.5	9.6		50.0	14.1		27.5	9.6	↓	67.5	9.6	
P CB1-14	*Pseudomonas rhodesiae*	45.0	17.3		37.5	5.0		35.0	10.0	↓	17.5	12.6		77.5	9.6		47.5	17.1		70.0	21.6		55.0	10.0	
P MF1-1	*Pseudomonas* sp.	60.0	16.3	↑	67.5	26.3	↑	80.0	8.2		17.5	9.6		92.5	9.6		82.5	20.6	↑	97.5	5.0		52.5	9.6	
PCB1 23	*Rhizobium lemnae*	67.5	12.6	↑	40.0	0.0		55.0	12.9	↓	5.0	10.0	↓	97.5	5.0	↑	50.0	0.0		92.5	9.6		40.0	28.3	
PM1 7	*Rhizobium lemnae*	65.0	5.8	↑	45.0	17.3		70.0	18.3		50.0	18.3	↑	80.0	14.1		67.5	18.9		90.0	8.2		82.5	5.0	
PM1 9	*Rhizobium lemnae*	30.0	14.1		37.5	9.6		70.0	8.2		27.5	15.0		90.0	11.5		52.5	17.1		90.0	8.2		52.5	5.0	
PK4 5	*Rhizobium lemnae*	37.5	9.6		32.5	15.0		15.0	5.8	↓	15.0	23.8		87.5	12.6		47.5	20.6		80.0	8.2		30.0	29.4	↓
PCB1 22	*Rhizobium rosettiformans*	30.0	11.5		52.5	17.1		52.5	5.0	↓	37.5	17.1		90.0	8.2		62.5	15.0		90.0	8.2		82.5	12.6	↑
PCB1 4	*Sinorhizobium fredii*	22.5	12.6		47.5	9.6		40.0	14.1	↓	22.5	17.1		87.5	5.0		70.0	8.2		92.5	9.6		60.0	31.6	
PCB1 5	*Sinorhizobium fredii*	62.5	5.0	↑	37.5	12.6		37.5	5.0	↓	40.0	14.1		90.0	8.2		47.5	9.6		72.5	12.6		70.0	11.5	
P CB1-12	*Stenotrophomonas rhizophila*	37.5	17.1		40.0	8.2		62.5	20.6		7.5	9.6	↓	85.0	5.8		50.0	11.5		90.0	11.5		47.5	20.6	
P CB1-16	*Stenotrophomonas rhizophila*	62.5	9.6	↑	50.0	14.1		60.0	11.5		12.5	5.0		92.5	5.0		55.0	19.1		90.0	8.2		62.5	9.6	
P CB1-17	*Stenotrophomonas rhizophila*	67.5	25.0	↑	47.5	9.6		50.0	11.5	↓	27.5	18.9		85.0	12.9		47.5	5.0		72.5	12.6		75.0	10.0	

Table D.4. Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from lentil roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, $P \leq 0.05$.

Isolate	Classification	Day 2												Day 8									
		canola			wheat			pea			lentil			canola			wheat			pea			lentil		
		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D		$\bar{x}$	S.D	
control		32.5	5.0		45.0	12.9		80.0	11.5		30.0	11.5		82.5	9.6		62.5	9.6		95.0	5.8		62.5	12.6	
L K1-1	*Bacillus halosaccharovorans*	70.0	16.3	↑	22.5	11.5	↓	60.0	18.3		22.5	17.3		92.5	9.6		42.5	12.6		90.0	10.0		65.0	20.8	
L K1-4	*Bacillus halosaccharovorans*	65.0	17.3		45.0	17.1		77.5	5.0		52.5	12.9	↓	92.5	9.6		50.0	12.6		92.5	5.0		82.5	17.1	
L K1-10	*Bacillus halosaccharovorans*	45.0	12.9		55.0	12.9		65.0	12.9		25.0	17.3		85.0	12.9		67.5	9.6		80.0	8.2		75.0	17.3	
L K1-15	*Bacillus halosaccharovorans*	72.5	9.6	↑	50.0	17.1		77.5	21.6		50.0	9.6	↑	85.0	5.8		55.0	20.6		90.0	8.2		87.5	9.6	
L K1-20	*Bacillus halosaccharovorans*	60.0	21.6		55.0	5.8		70.0	11.5		27.5	20.6		85.0	10.0		62.5	12.6		90.0	14.1		77.5	9.6	
L K1-24	*Bacillus halosaccharovorans*	20.0	16.3		65.0	15.0	↑	72.5	12.6		27.5	17.3		87.5	9.6		70.0	8.2		85.0	5.0		87.5	8.2	↑
LCB1 13	*Bordetella hinzii*	70.0	11.5		27.5	22.2		70.0	8.2		27.5	9.6		92.5	5.0		35.0	23.8		87.5	9.6		65.0	25.2	
LSV2 8	*Methylobacterium populi*	90.0	14.1	↑	50.0	14.1		62.5	12.9		35.0	12.9		97.5	5.0	↑	57.5	12.9		72.5	15.0		67.5	9.6	
LSV2 1	*Microbacterium murale*	42.5	12.6		50.0	11.5		55.0	17.3		30.0	8.2		90.0	0.0		60.0	11.5		75.0	10.0		67.5	9.6	
L SV2-2	*Microbacterium murale*	67.5	15.0		40.0	18.3		65.0	12.9		27.5	9.6		92.5	5.0		52.5	9.6		92.5	9.6		72.5	17.1	
L SV2-3	*Microbacterium murale*	85.0	5.8	↑	35.0	15.0		47.5	5.0		30.0	5.8		92.5	5.0		47.5	12.9		75.0	9.6		87.5	10.0	↑
L SV2-5	*Microbacterium murale*	82.5	9.6	↑	32.5	27.5		60.0	8.2		5.0	12.9	↓	87.5	5.0		42.5	12.9		95.0	5.0		60.0	17.1	
L SV2-6	*Microbacterium murale*	87.5	9.6	↑	47.5	12.6		75.0	25.0		30.0	15.0		95.0	5.8	↑	50.0	9.6		95.0	5.8		62.5	9.6	
L SV2-14	*Microbacterium murale*	60.0	8.2		52.5	15.0		45.0	12.9		22.5	17.1		92.5	5.0		67.5	12.6		72.5	22.2		82.5	9.6	
L SV2-15	*Microbacterium murale*	57.5	20.6		57.5	17.1		42.5	9.6	↓	22.5	9.6		85.0	10.0		67.5	12.6		80.0	11.5		70.0	18.3	
L SV2-18	*Microbacterium murale*	80.0	8.2	↑	47.5	14.1		67.5	8.2		22.5	8.2		92.5	5.0		52.5	15.0		87.5	11.5		72.5	17.1	
L SV2-17	*Microbacterium mangrovi*	57.5	17.1		55.0	12.9		82.5	17.1		40.0	16.3		85.0	10.0		70.0	16.3		90.0	8.2		95.0	10.0	↑
L K1-21	*Paenibacillus pabuli*	72.5	12.6	↑	62.5	8.2		60.0	22.2		35.0	11.5		92.5	5.0		77.5	17.1		77.5	11.5		75.0	20.8	
L CB1-3	*Pantoea agglomerans*	87.5	9.6	↑	52.5	9.6		60.0	5.8		45.0	14.1		92.5	5.0		65.0	8.2		80.0	5.8		80.0	22.2	
L CB1-10	*Pantoea agglomerans*	67.5	15.0		42.5	15.0		55.0	5.8		32.5	12.6		80.0	8.2		62.5	12.6		77.5	20.6		67.5	5.0	
L CB1-14	*Pantoea agglomerans*	77.5	9.6	↑	40.0	17.3		75.0	26.3		40.0	9.6		90.0	8.2		50.0	14.1		95.0	9.6		60.0	5.0	

Table D.4. (cont.). Seed germination (%) of canola, wheat, pea and lentil inoculated with bacterial endophytes isolated from lentil roots, determined at 2 and 8 days after inoculation. Asterisks indicate significant differences (↑ increase or ↓ decrease) compared with control; Dunnett, $P \leq 0.05$.

Isolate	Classification	Day 2								Day 8							
		canola		wheat		pea		lentil		canola		wheat		pea		lentil	
		$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D	$\bar{x}$	S.D
L CB1-9	*Pseudomonas brassicacearum*	67.5	12.6	27.5	9.6	25.0	12.9 ↓	25.0	12.9	70.0	14.1	30.0	8.2 ↓	32.5	5.0 ↓	37.5	20.6
L CB1-2	*Pseudomonas brassicacearum*	67.5	9.6	40.0	14.1	50.0	14.1	10.0	8.2	70.0	8.2	40.0	14.1	60.0	14.1 ↓	47.5	17.1
L CB1-6	*Pseudomonas brassicacearum*	75.0	5.8 ↑	47.5	0.0	30.0	25.0 ↓	22.5	12.6	87.5	12.6	50.0	10.0	42.5	32.7 ↓	65.0	5.0
L CB1-7	*Pseudomonas tolaasii*	30.0	8.2	35.0	10.0	32.5	12.6 ↓	20.0	14.1	70.0	8.2	45.0	12.9	32.5	12.6 ↓	65.0	10.0
LK1 12	*Rhizobium leguminosarum*	87.5	15.0 ↑	47.5	15.0	42.5	5.0 ↓	37.5	5.8	97.5	5.0 ↑	57.5	12.9	70.0	9.6	65.0	10.0
LK1 13	*Rhizobium leguminosarum*	55.0	10.0	32.5	5.0	45.0	5.8	30.0	21.6	92.5	9.6	50.0	8.2	75.0	17.3	57.5	22.2
LK1 14	*Rhizobium leguminosarum*	67.5	15.0	40.0	16.3	65.0	12.9	37.5	17.1	87.5	9.6	45.0	19.1	80.0	8.2	62.5	22.2
LK1 19	*Rhizobium leguminosarum*	45.0	19.1	62.5	15.0	57.5	5.0	25.0	5.8	90.0	8.2	65.0	12.9	67.5	9.6	65.0	10.0

APPENDIX E: Effect of endophytic bacterial strains inoculation on canola and wheat grown in agricultural soils from Central Butte, Saskatchewan

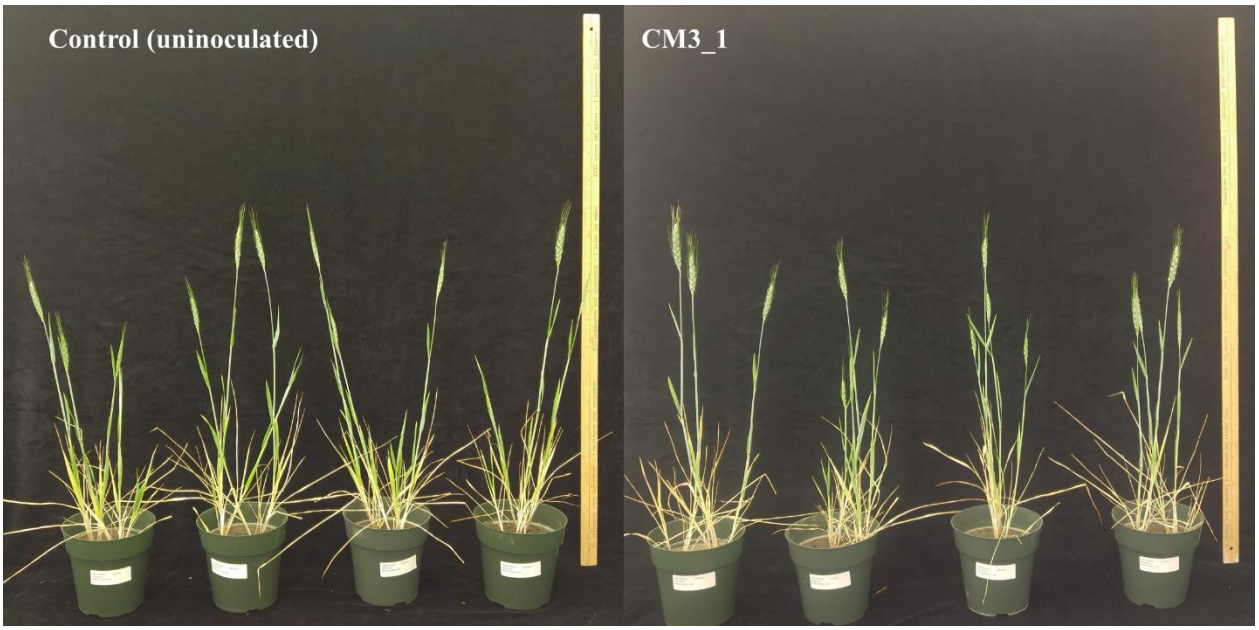

Fig. E.1. Effect of *Stenotrophomonas maltophilia*, strain CM3_1 inoculation on wheat grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

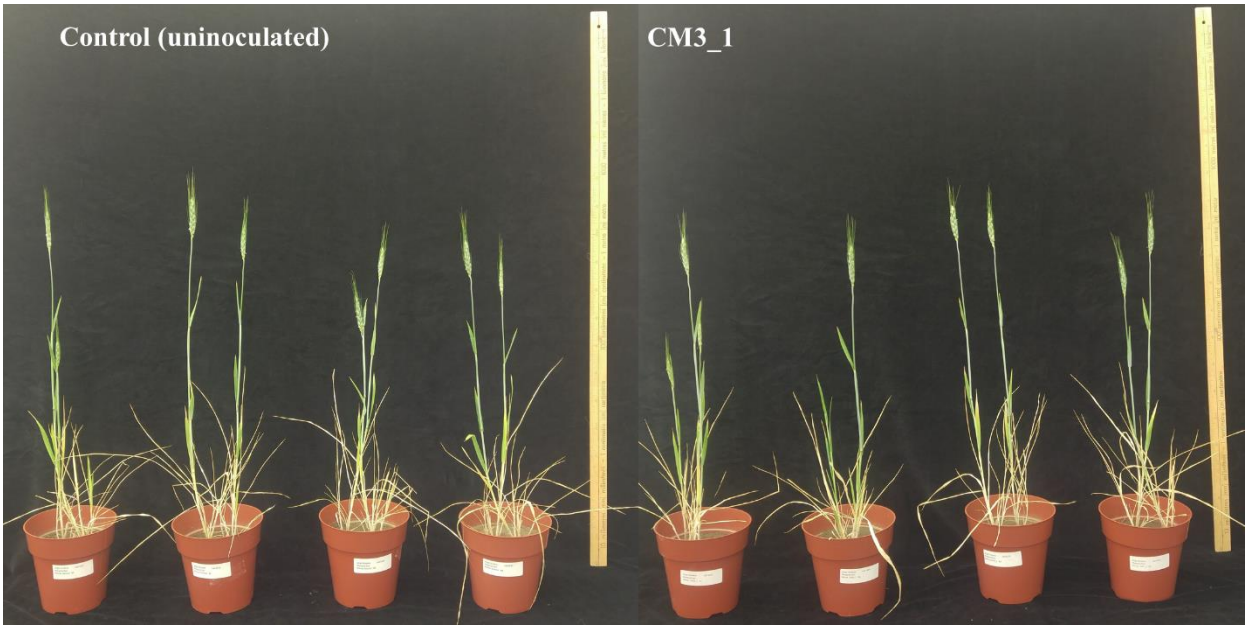

Fig. E.2. Effect of *Stenotrophomonas maltophilia*, strain CM3_1 inoculation on wheat grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

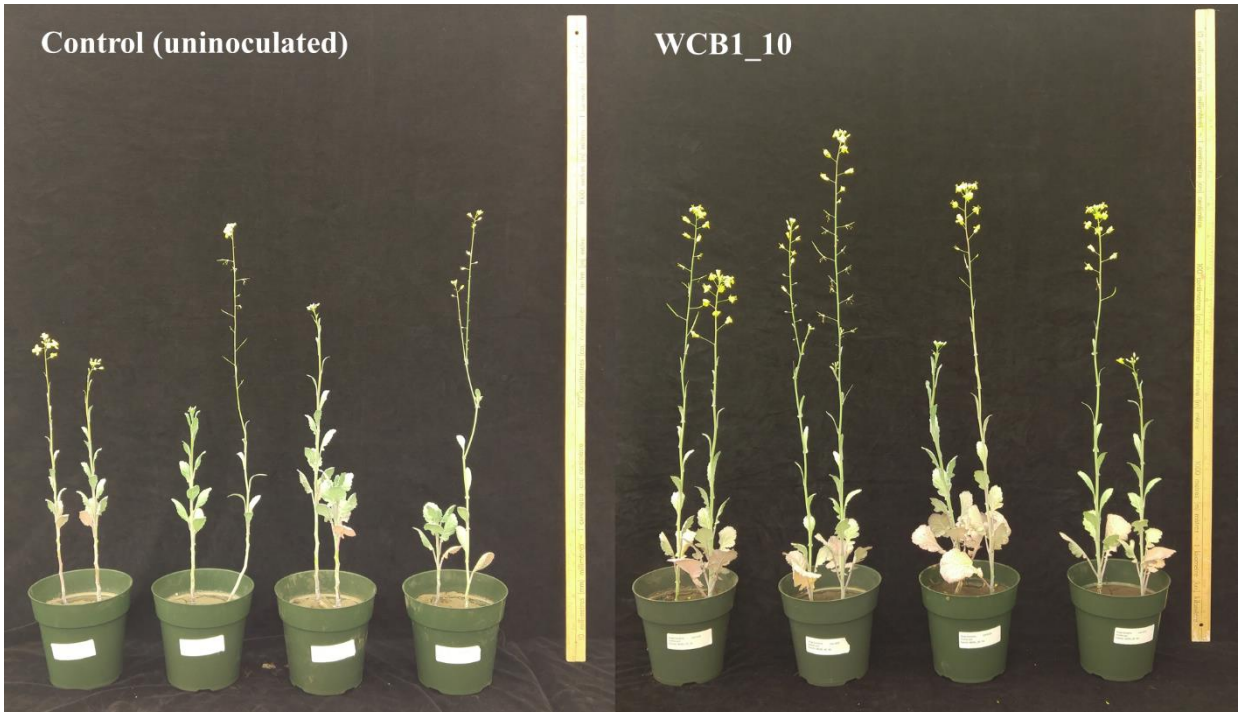

Fig. E.3. Effect of *Rhodococcus cerastrii*, strain WCB1_10 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

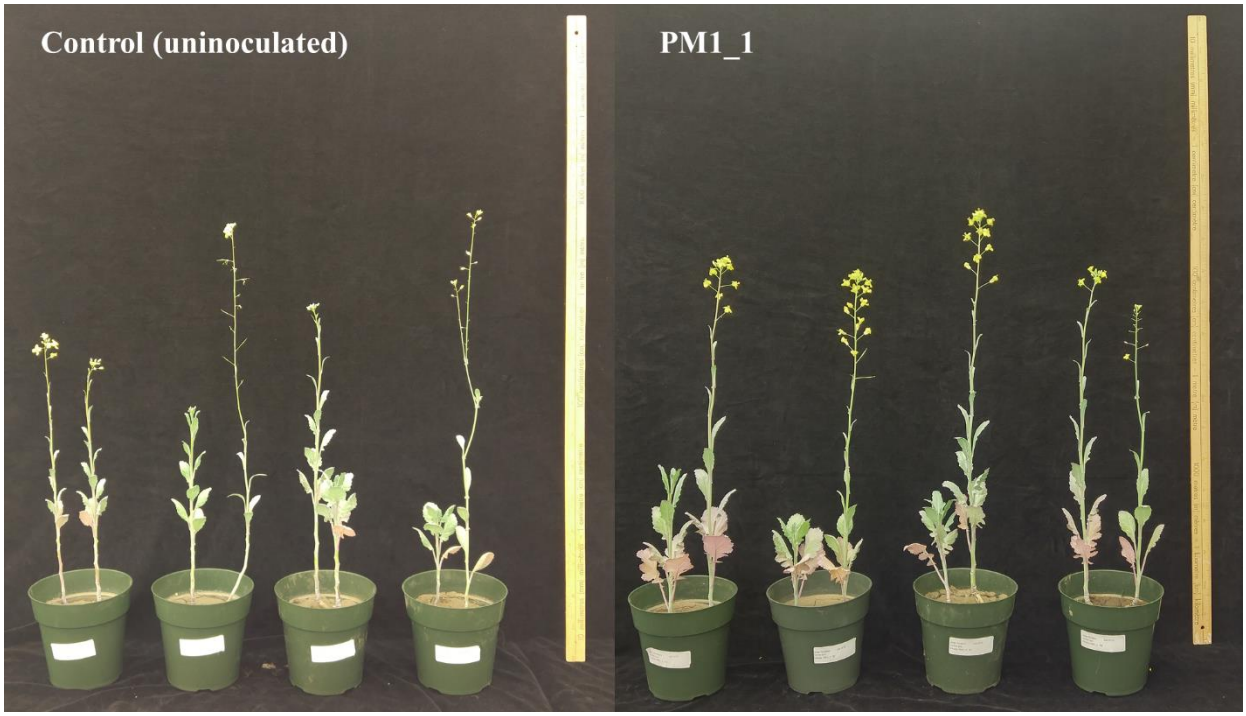

Fig. E.4. Effect of *Pseudomonas* sp., strain PM1_1 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

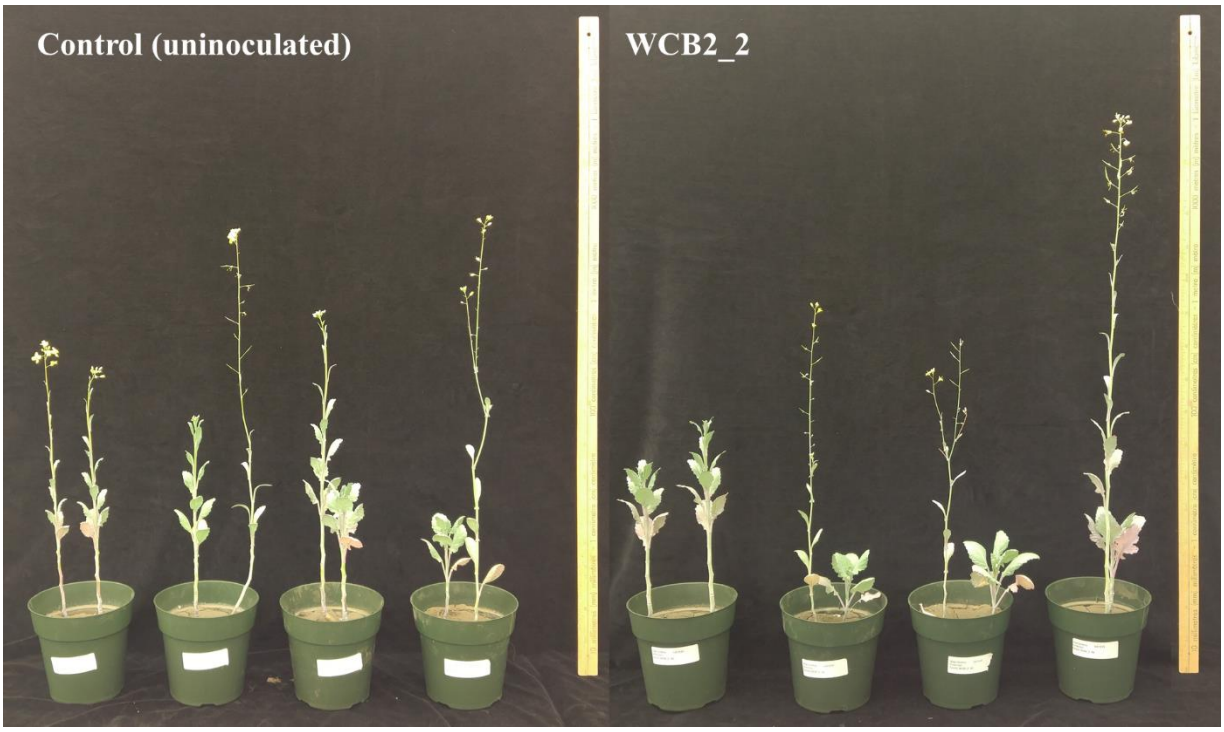

Fig. E.5. Effect of *Paenibacillus taohuashanense*, strain WCB2_2 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil A). Plants were harvested at flowering.

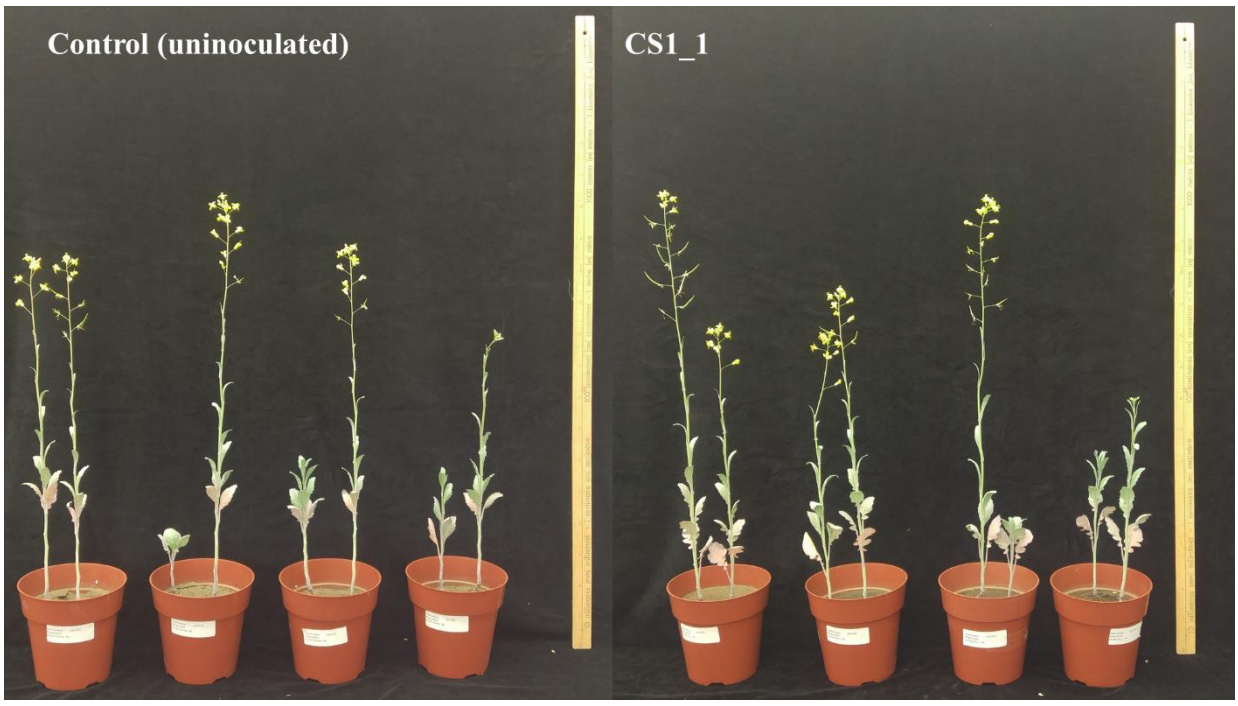

Fig. E.6. Effect of *Pantoea vagans*, strain CS1_1 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

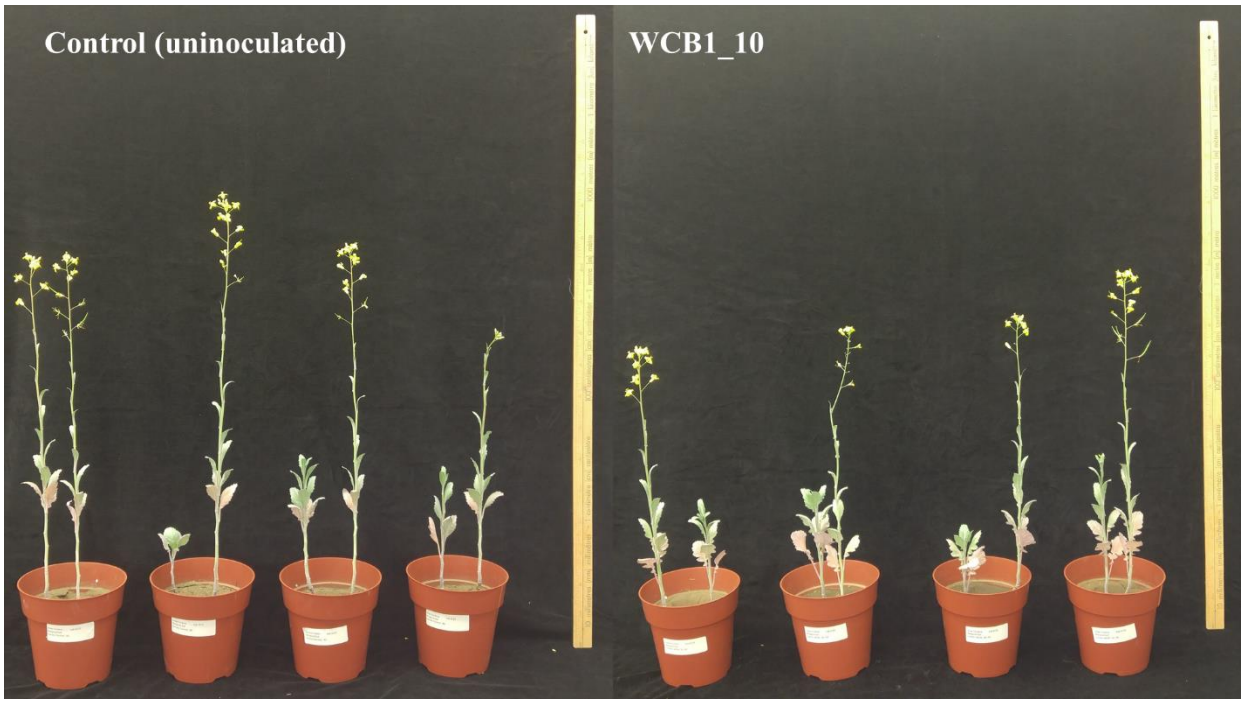

Fig. E.7. Effect of *Rhodococcus cerastrii*, strain WCB1_10 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

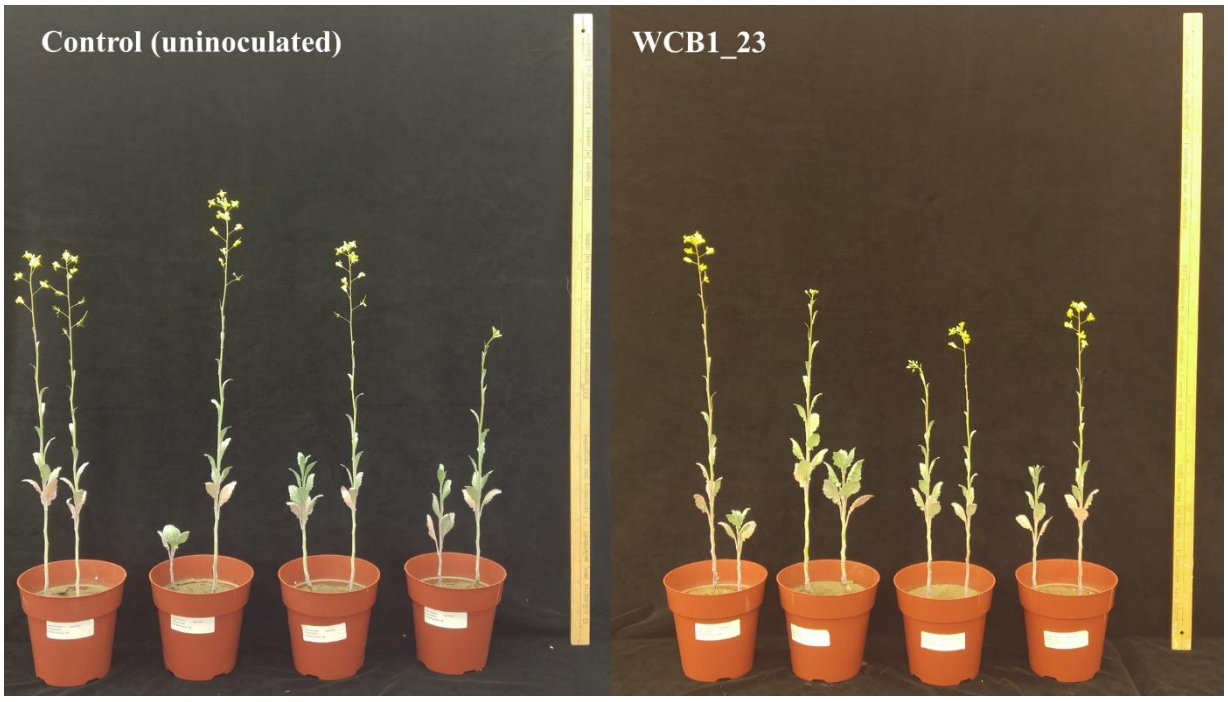

Fig. E.8. Effect of *Agroccoccus carbonis*, strain WCB1_23 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

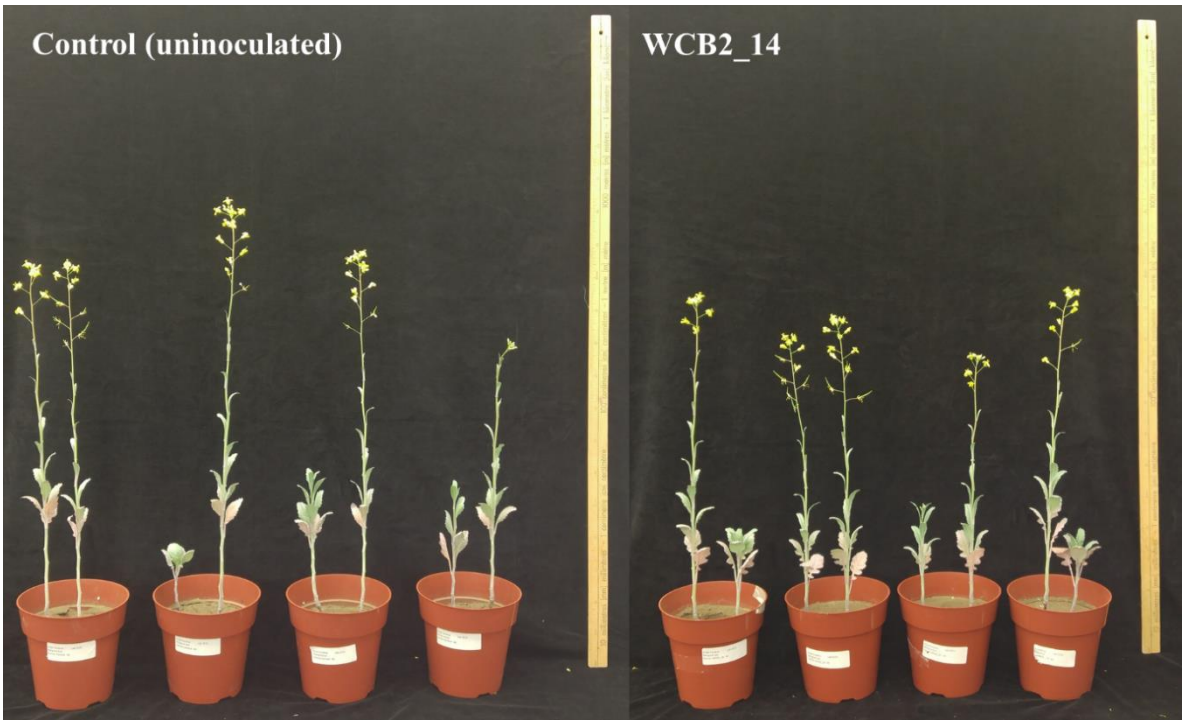

Fig. E.9. Effect of *Stenotrophomonas rhizophila*, strain WCB2_14 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

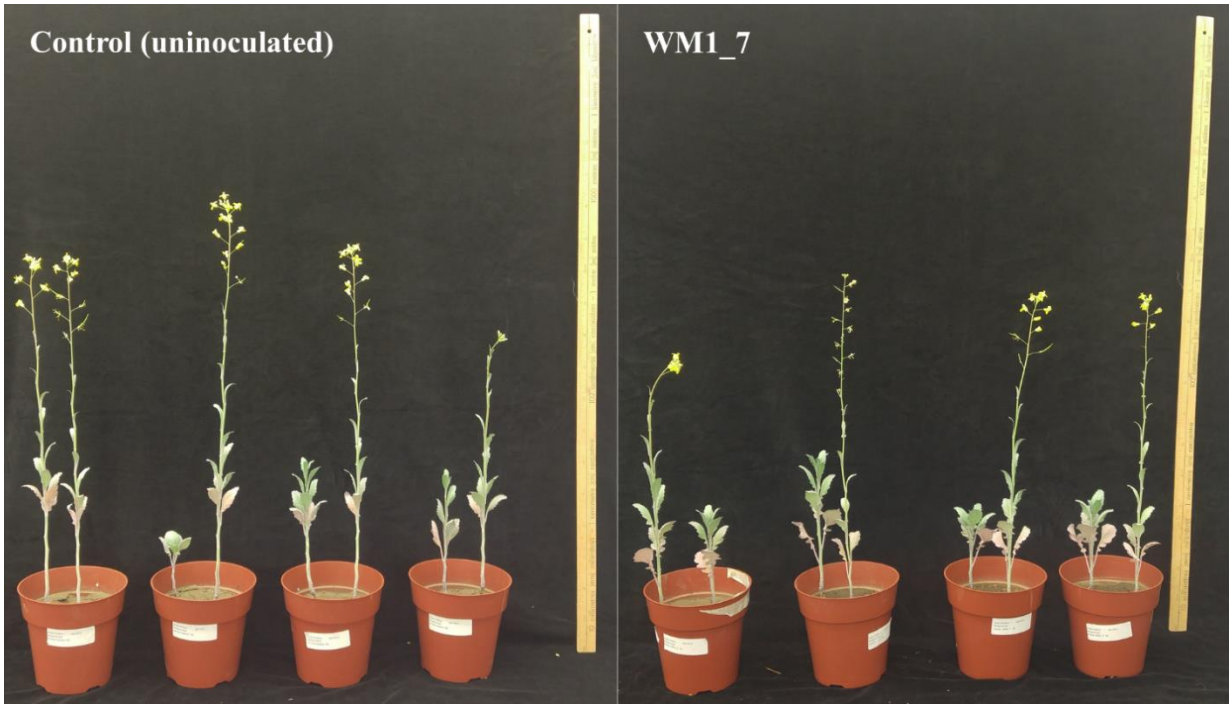

Fig. E.10. Effect of *Leifsonia xyli*, strain WM1_7 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

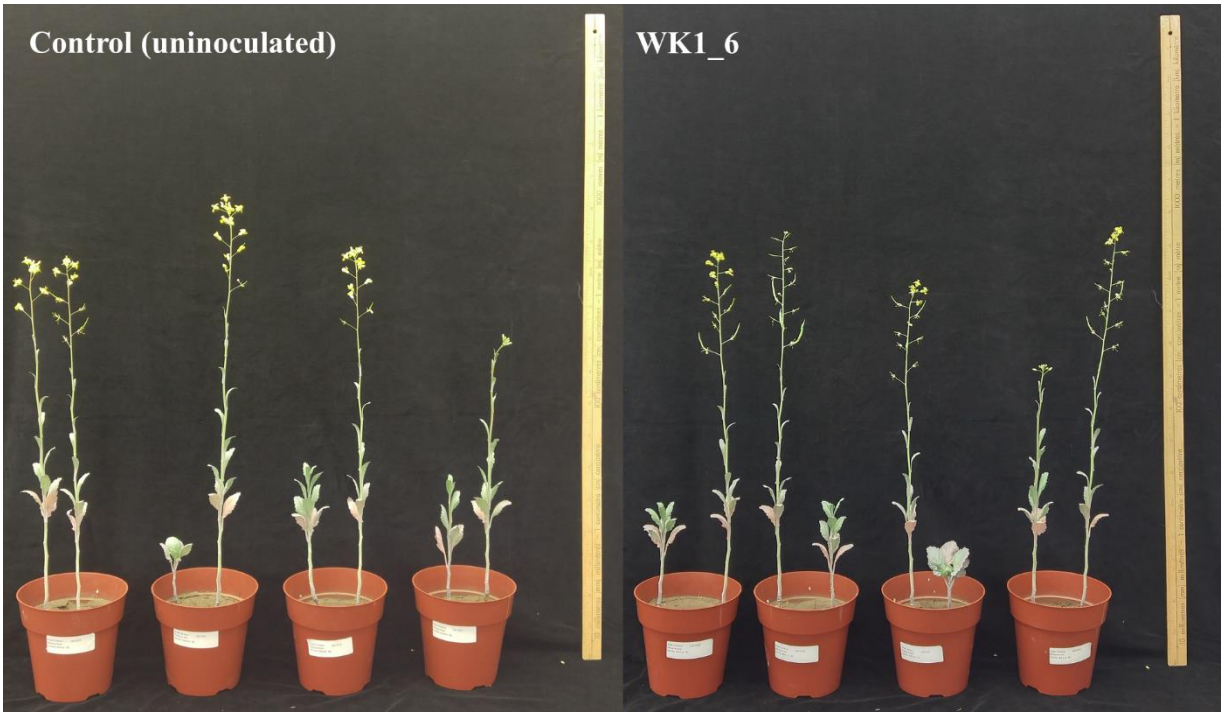

Fig. E.11. Effect of *Xanthomonas fuscans*, strain WK1_6 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

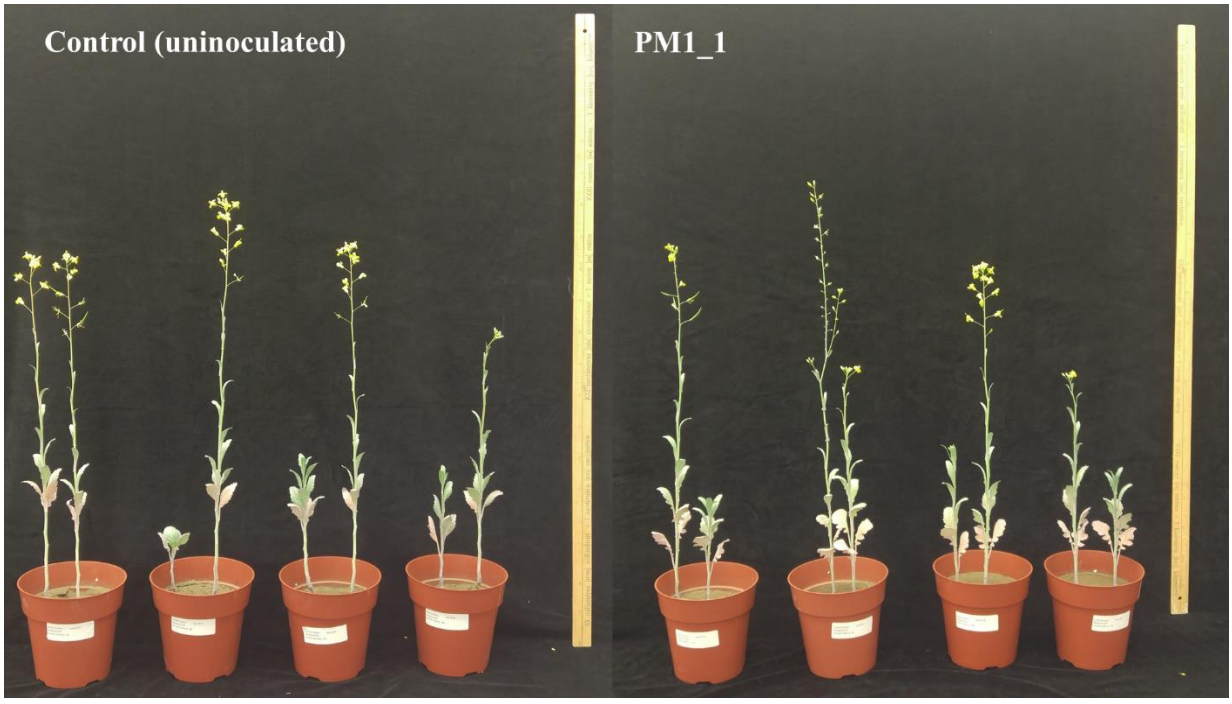

Fig. E.12. Effect of *Pseudomonas* sp., strain PM1_1 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.

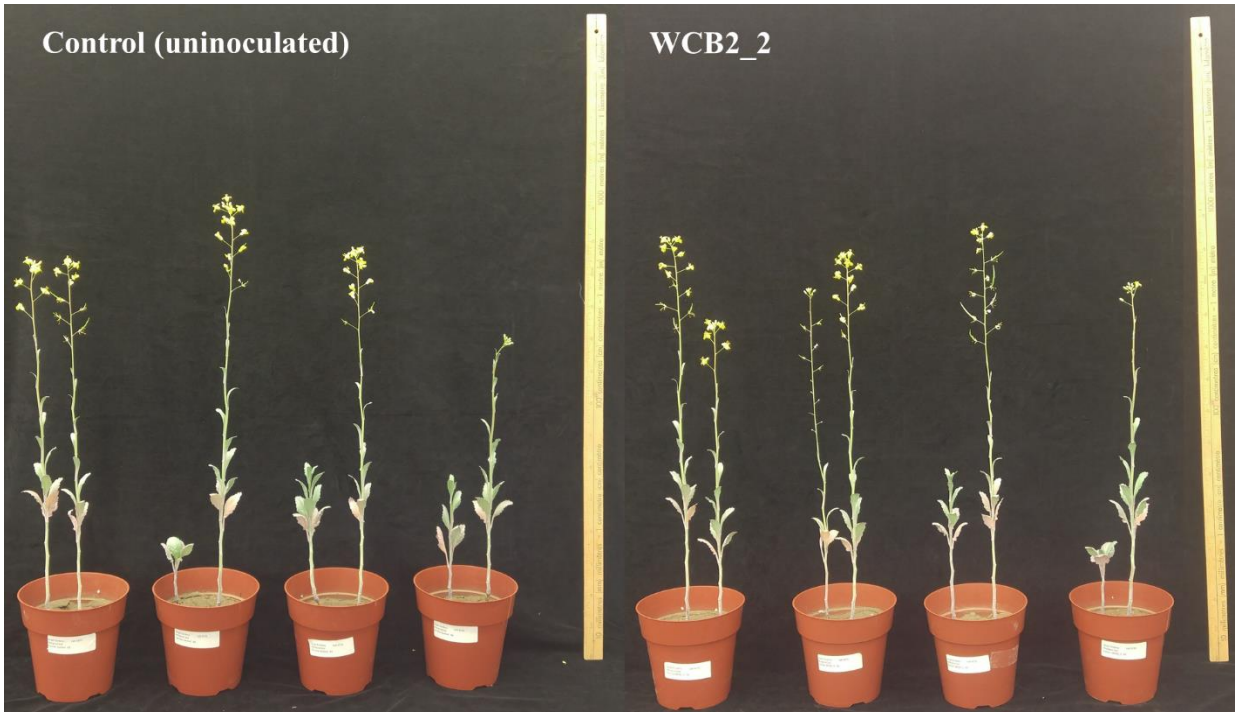

Fig. E.13. Effect of *Paenibacillus taohuashanense*, strain WCB2_2 inoculation on canola grown in an agricultural potted soil from Central Bute, Saskatchewan (soil B). Plants were harvested at flowering.